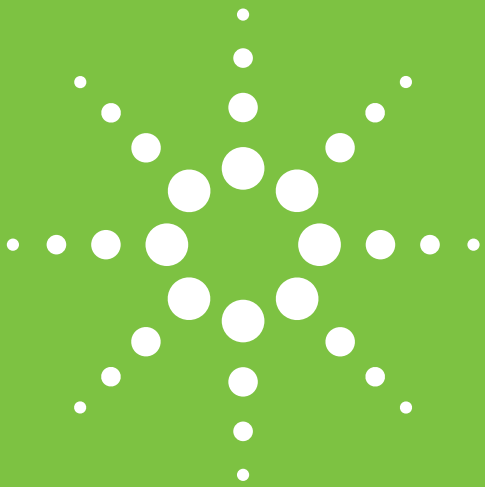
Agilent 2100 Bioanalyzer

Application Compendium

DNA, RNA, PROTEIN AND CELL ANALYSIS

The Measure of Confidence

Agilent Technologies



Agilent 2100 Bioanalyzer

Application
Compendium
DNA, RNA, PROTEIN AND CELL ANALYSIS

Agilent Technologies is a leading global provider of life science and chemical analysis solutions, including instrumentation, supplies, software and services. In Life Sciences, Agilent helps investigators advance basic research, unlock the genetic causes of disease and accelerate the discovery and development of new drugs. Agilent's life sciences business addresses the needs of academic, institutional and pharmaceutical scientists in five key areas:

- Genomics
- Proteomics
- Metabolomics
- Bioinformatics
- Pharmaceutical analysis

Let us provide you with the right solution for your success.

The Agilent 2100 Bioanalyzer is an easy-to-use benchtop system with ready-to-run kits for a wide range of applications.

- **On-chip flow cytometry**
  The easy way to acquire dual-color, cell-based fluorescence data
- **DNA size and quantity**
  High resolution separation and quantification of DNA down to pg/µL sensitivity
- **RNA quality check with RIN**
  The industry standard for RNA analysis offering total RNA, mRNA and Small RNA's data including RIN algorithm (RNA Integrity Number)
- **SDS-PAGE replacement for protein analysis**
  The fast and reliable way to determine the quantity and purity of proteins from Coomassie down to silver stain sensitivity

When combined with any one of our kits, you will discover how, lab-on-a-chip technology can revolutionize your laboratory.

Microfluidics utilizes interconnected networks of micro-channels and wells for the analysis of various sample types. The technology reduces overall space and volume requirements and allows online integration of many workflow steps like sample enrichment, separation, staining, de-staining, and detection. Advantages of microfluidics therefore include dramatically reduced sample and reagent consumption, significantly faster analysis time and less hands-on activities during sample preparation and data analysis. The Agilent 2100 Bioanalyzer represents the first microfluidic lab-on-a-chip system for the electrophoretic analysis of DNA, RNA, and proteins and the flow-cytometric analysis of cells. This versatility makes the Bioanalyzer an indispensable tool for the molecular biologist and biochemist.

The Agilent 2100 Bioanalyzer with lab-on-a-chip technology will increase the quality and efficiency of your analysis enhancing the productivity of your day.

Lab-on-a-chip technology has many advantages over conventional techniques. These advantages include improved data precision and reproducibility, short analysis times, minimal sample consumption, improved automation and integration of complex workflows.

One of the many benefits the Agilent 2100 Bioanalyzer has over conventional bioanalytical methods is the elimination of time consuming procedures – you enjoy standardized handling and interpretation of data. It simplifies the process of data gathering and analysis down to three quick and easy steps:

Load sample, run analysis, view data.

- The Agilent 2100 Bioanalyzer and the various **DNA kits** are the tools of choice for automated sizing and quantification of products generated by RT-PCR and any type of multiplex PCR with unprecedented accuracy and reproducibility. It not only provides the detection of the presence or absence of a PCR product, but also offers quantification of this product and detection of unspecific amplification. The Agilent 2100 Bioanalyzer therefore helps to optimize PCR reactions for gene expression, sequencing, cloning and typing. The DNA 12000 kit also offers the accurate analysis of restriction digests.
- The **High Sensitivity DNA kit** provides sizing and quantification of DNA fragments and DNA smears in the 50 to 7000 bp size range down to pg/µL sensitivity. This is especially useful for sample quality control and the monitoring of critical steps in next-generation sequencing (NGS) workflows, including DNA fragmentation, target enrichment, and DNA library amplification.
- Due to the omnipresence of RNases, and the instability of RNA, integrity checks and sample quantification are essential steps before any RNA dependent application. The 2100 Expert software generates the unambiguous RNA Integrity Number (**RIN**), a quantification estimate, calculates ribosomal ratios of total RNA samples and automatically detects ribosomal RNA contamination in mRNA. The **RNA 6000 Nano kit** is the industry standard for RNA sample QC. The **RNA 6000 Pico kit** allows to catch RNA degradation with sample amounts as low as 200 pg of total RNA.
- The high resolution **Small RNA kit** allows separation, verification and optimization of miRNA after extraction procedures. By consuming only 1 µL sample even pg amounts of purified small RNA can be measured reproducibly and comparably within 30 minutes. By effectively staining single- and double-stranded oligonucleotides at the same time, the assay is a versatile tool.
- The **Protein 80 and Protein 230 kits** provide a fast and easy way to analyze a wide range of samples, whether expressing recombinant proteins, purifying proteins, performing stability studies or checking antibody quality. The on-chip electrophoresis provides size, purity and concentration information for ten protein samples in less than 30 minutes. The lab-on-a-chip approach eliminates handling SDS-PAGE gels, staining or imaging steps.

- With the **High Sensitivity Protein 250 kit** it is possible to analyze proteins down to 1 pg/µL (on chip), which is equivalent or superior to silver stain SDS-PAGE. It provides quantification over a dynamic range of up to 4 orders of magnitude with the reproducibility and ease-of-use only associated with the Agilent 2100 Bioanalyzer.
- The **Flow Cytometry set** and the **Cell kit** for the Agilent 2100 Bioanalyzer allows scientists to perform simple on-chip flow cytometry assays, thereby making the 2100 Bioanalyzer the industry's only platform able to run RNA, DNA, proteins and cell analysis. Six cell samples each 10 µL with 20,000 prestained cells are loaded onto the chip and the fluorescence intensities in two channels for about 750 single cells per sample are measured within 25 minutes.
- The optional **Agilent 2100 Security Pack software** ensures full **21 CFR Part 11 compliance** of your 2100 Bioanalyzer system for regulated environments such as pharmaceutical QA/QC labs or manufacturing, addressing requirements such as electronic signatures, audit trails and user authentication. Along with **IQ and OQ/PV** support services and Declarations of Conformity for all components offered for all assays and kits, your Agilent 2100 Bioanalyzer system will be compliant in no time.

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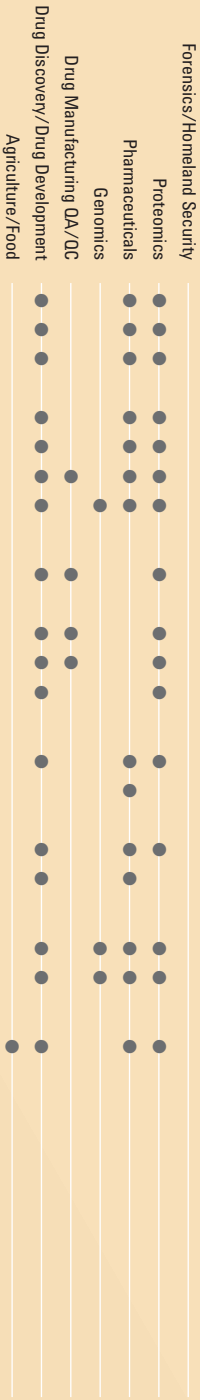
I. Cell fluorescence analysis



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- Cell surface antibody staining – CD4 in CCRF CEM T-cells
- Cell surface antibody staining – CD3 in T-cell leukemia
- CD3 expression in T-cell leukemia via on-chip staining
- Intracellular glucocorticoide receptor (GR) antibody staining in H4 hepatocytes
- Analyzing a limited number of cells
- Baculovirus titre determination
- Upregulated gene expression in primary cells

Transfection efficiency monitoring
- Green fluorescent protein in CHO cells
- On-chip staining of GFP expression for optimizing transfection conditions with different DNA:lipid ratios
- Verification of stable transfected cell clones by on-chip antibody staining
- Transfection of primary cells

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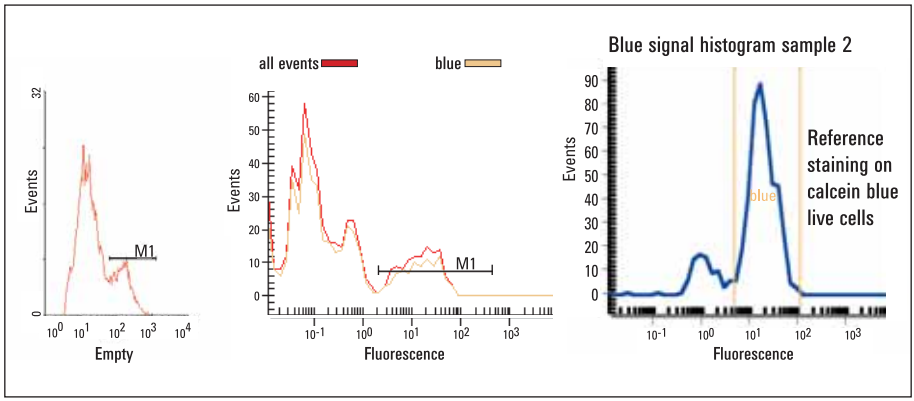
- siRNA transfection optimization
- Monitoring of gene silencing experiments

Cells – others
- Identification and counting of blood cells in whole blood samples

Protein expression monitoring

Cell surface antibody staining – CD4 in CCRF CEM T-cells

Flow cytometer (10,000 events) 2100 Bioanalyzer (500 events)



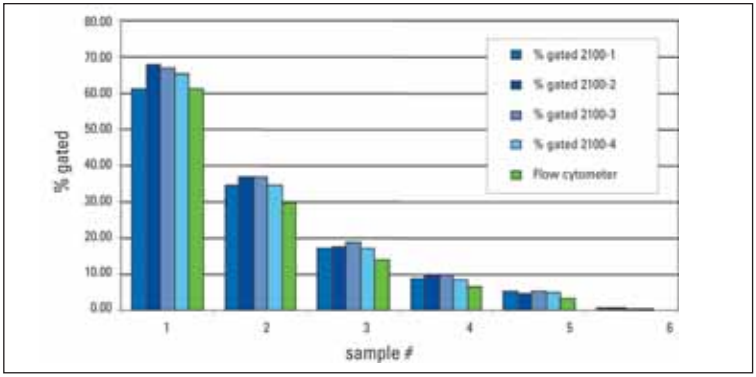
Kit: Cell kit
Assay: Antibody staining assay
Application: CCRF-CEM cells were stained with hCD4-APC labeled antibodies and calcein live dye. 65 % of all CCRF-CEM live cells (yellow curve) are expressing CD4 protein which is good in comparison to conventional flow cytometer results.

Application note: 5988-4322EN

Protein expression monitoring

Cell surface antibody staining – CD3 in T-cell leukemia

Averaged data per instrument



Mean % CD3+ cells				
2100-1	2100-2	2100-3	2100-4	Flow cyt.
60.9	67.8	66.6	65.0	60.9
34.4	36.7	36.7	34.3	29.8
17.3	17.6	18.7	17.2	13.8
8.9	9.4	9.9	8.3	6.5
5.1	4.4	5.3	4.9	3.2
0.8	0.6	0.3	0.3	0.0

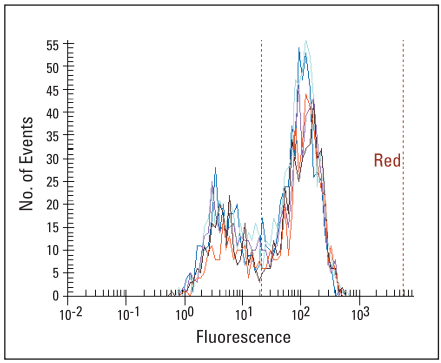
Kit: Cell kit
Assay: Antibody staining assay
Application: Jurkat (T-cell leukemia) cells were stained with calcein alone or with calcein and APC-labeled anti-CD3 antibody. To mimic different subpopulation sizes, mixtures of both populations were prepared at various ratios.
Samples were analyzed with 4 2100 Bioanalyzer instruments on 5 chips and compared to a flow cytometer reference instrument. Interestingly, small subpopulations (like 10 – 20 %) could be analyzed with good accuracy and reproducibility.

Application note: 5988-4322EN

Protein expression monitoring

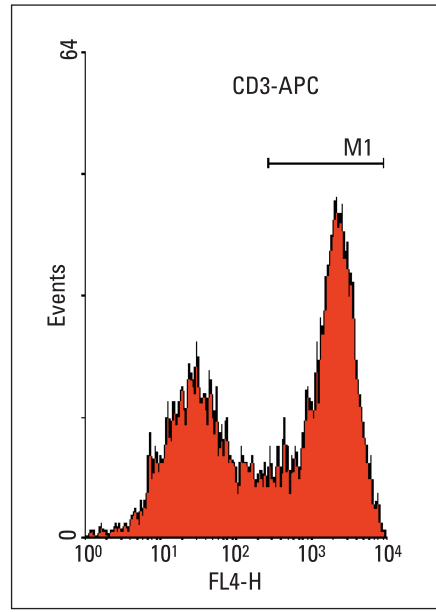
CD3 expression in T-cell leukemia via on-chip staining

A. On-chip 2100 Bioanalyzer



Sample	% of Gated	Sample	% of Gated
1	-	4	64.6
2	66.9	5	66.7
3	67.2	6	72.0

B. Conventional flow cytometry



Kit: Cell kit
Assay: Antibody staining assay
Application: Jurkat cells were stained on-chip with anti hCD3-APC prediluted 1:5.5 in cell buffer and Calcein (1:50 in cell buffer). After an incubation time of 25 minutes in the chip, samples were measured in the 2100 Bioanalyzer. The faster and easier on-chip staining procedure has the advantage here of reducing cell consumption 17 fold and antibody reagent costs 80 fold.

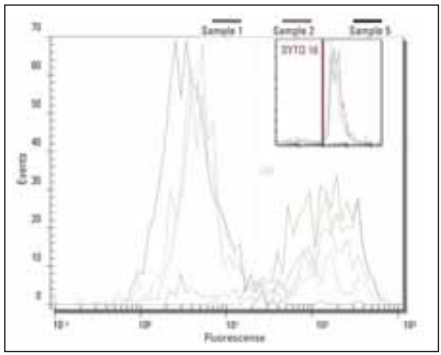
A) Overlay of representative histograms of calcein and antibody treated cells.
B) Comparison between on-chip staining data and data obtained by measuring cells stained by conventional staining on a flow cytometer.

Application note: 5988-7111EN

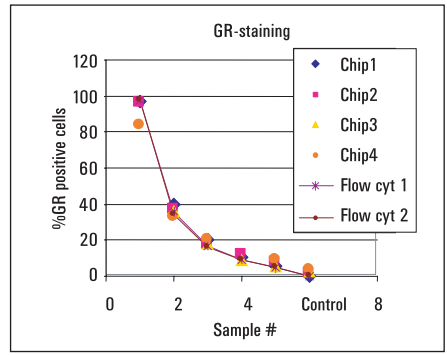
Protein expression monitoring

Intracellular glucocorticoide receptor (GR) antibody staining in H4 hepatocytes

Chip histogram overlay from 700 cells/sample



Correlation of chip vs. flow cytometer results

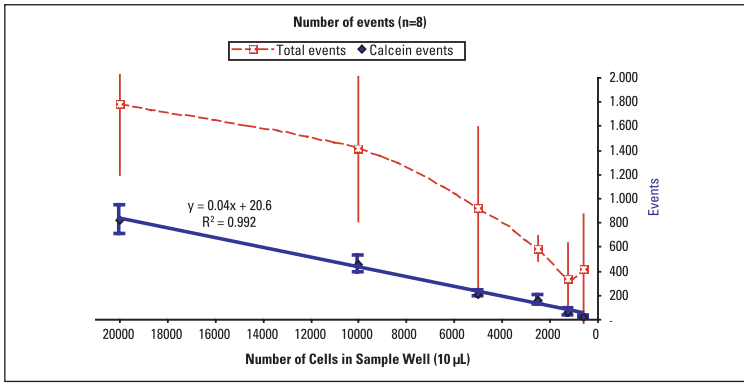


Kit: Cell kit
Assay: Generic assay
Application: H4 hepatocytes cells were stained with SYTO16 DNA dye alone or with SYTO16 and GR primary antibody. After washing, both cell preparations were stained with APC-labeled secondary antibody. Mixtures of both populations were prepared at various ratios.
The insert in the left picture shows the overlay of all six cell samples in the blue reference color. The black histogram represents data from the control sample, no GR detected.
All other 5 samples have significant staining above marked fluorescence intensity in the red.
Good chip to chip reproducibility and comparison to flow cytometer is demonstrated.

Application note: 5988-4322EN

Protein expression monitoring

Analyzing a limited number of cells



Cells	Live-CD3+	STD(n=4)
20,000	83.7 %	3.5 %
10,000	85.6 %	4.1 %
5,000	87.7 %	4.2 %
2,500	84.0 %	3.0 %
1,250	89.8 %	6.5 %
625	90.0 %	9.3 %

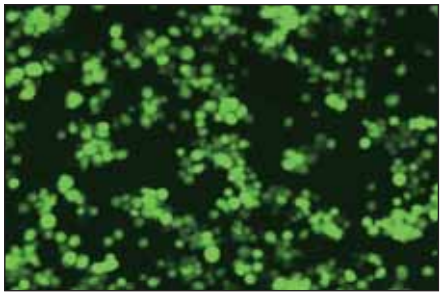
Kit: Cell kit
Assay: On-chip antibody staining assay
Application: The direct comparison of different input numbers of cells (down to 625 cells in 10 µL) for the on-chip staining protocol reveals that even with a much lower number than the recommended 20000 cells/10 µL for the standard protocol reliable and meaningful results can be achieved with good reproducibility. The data shown were generated with CD3-positive Jurkat cells stained with an anti-CD3 antibody for the CD3 protein and counterstained with the live cell stain Calcein AM. Similar results were obtained with primary human dermal fibroblasts (PHDF) indicating the usefulness of this method for scarce specimen. The lack of sensitivity, automation and convenient quantitation found with other methods can be circumvented easily by using the 2100 Bioanalyzer.

Application note: 5989-0746EN

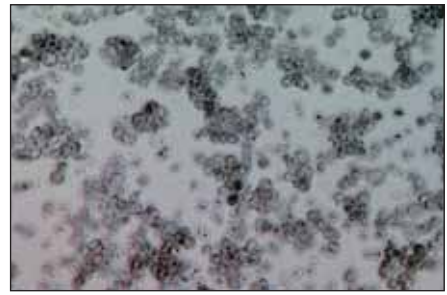
Protein expression monitoring

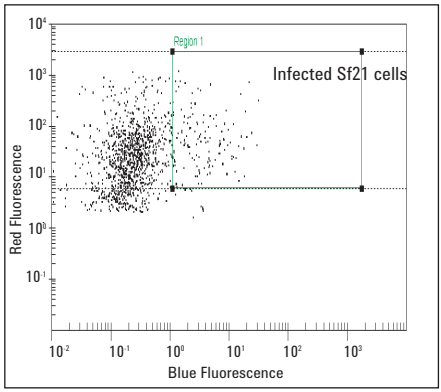
Baculovirus titre determination

Fluorescent Light



Transmitted Light





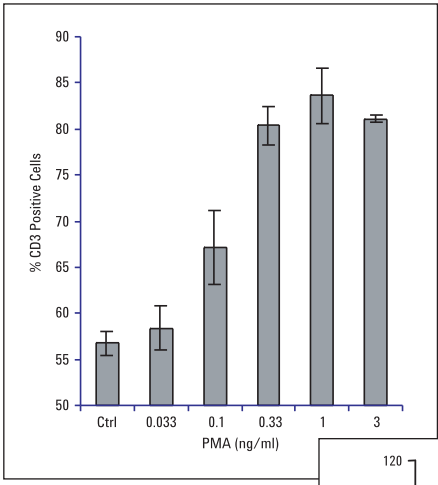
Kit: Cell kit
Assay: GFP Assay
Application: A fast and convenient method exists for the calculation of baculovirus titre for expression systems facilitating insect cells. Using GFP-linked co-expression plasmids, the 2100 Bioanalyzer and the flow cytometry set allows the calculation of the viral titre for six samples in approximately 90 minutes. It is superior to traditional plaque assays in terms of labor time, automation and user-to-user variability.

Application note: 5989-1644EN

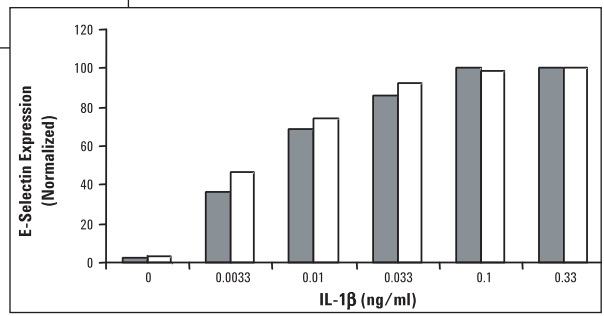
Protein expression monitoring

Upregulated gene expression in primary cells

A



B



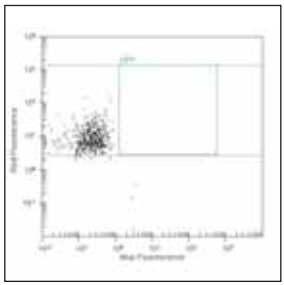
Kit: Cell kit
Assay: On-chip antibody staining assay
Application: Flow cytometric analysis of primary cells can present a challenge for researchers due to limited availability and life span of primary cells. A dose-respondent upregulation of protein expression in primary cells using only a minimum number of cells in a fast on-chip-staining approach is shown here. Activation of peripheral blood lymphocytes by phorbol-12-myristate-13-acetate (PMA) leads to increased expression of the T cell receptor CD3 (Figure A, mean from 3 experiments).
For HUVECs (human umbilical vein endothelia cells) the induction of E-selectin (CD62E) expression upon IL-1β treatment is shown (Figure B, white bars) in comparison to results from a conventional flow cytometer (white bars).

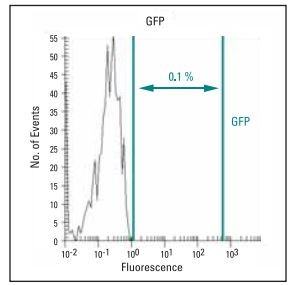
Application note: 5989-2718EN

Transfection efficiency monitoring

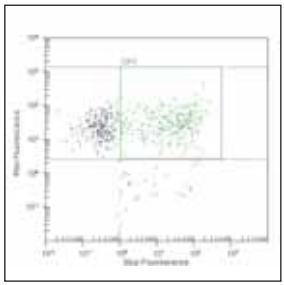
Green fluorescent protein in CHO cells

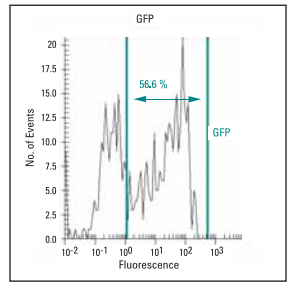
Mock transfected cells

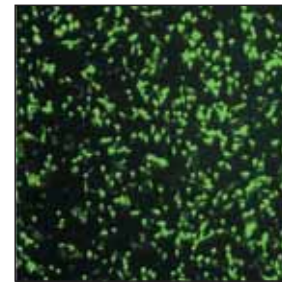




GFP transfected cells





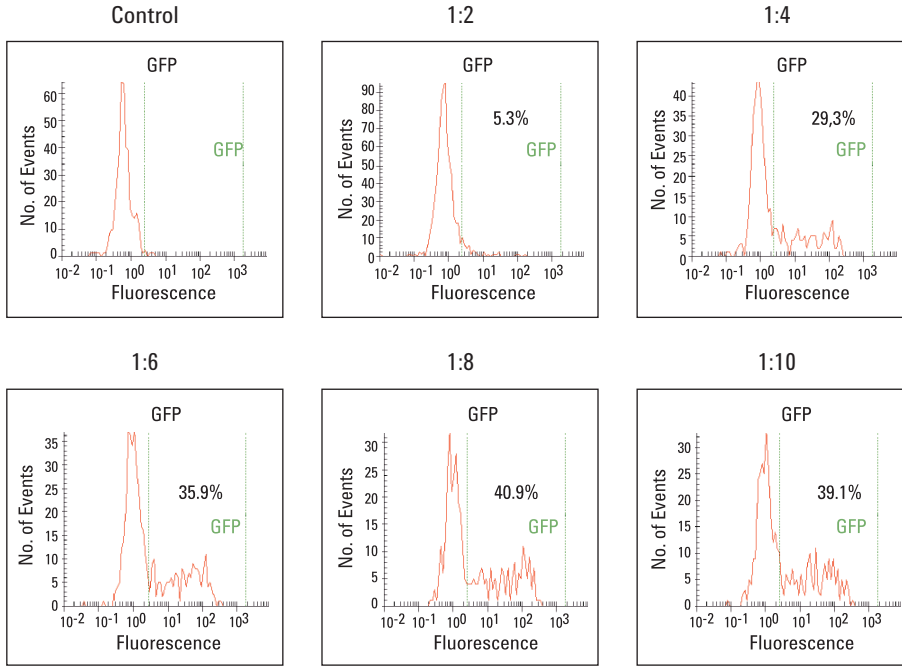


Kit: Cell kit
Assay: GFP assay
Application: Chinese hamster ovary (CHO-K1) cells were transfected with EGFP DNA by a lipofection method. The upper panel shows the control mock transfection; here cells don't express GFP. Examples for data evaluation in dotplot view and histogram view are shown in comparison to the microscopy view. For analysis on the 2100 Bioanalyzer, cells were stained with a red dye for live cells (reference stain). The transfection efficiency of 56 % can be easily determined with the 2100 Bioanalyzer.

Application note: 5988-4320EN

Transfection efficiency monitoring

On-chip staining of GFP expression for optimizing transfection conditions with different DNA:lipid ratios



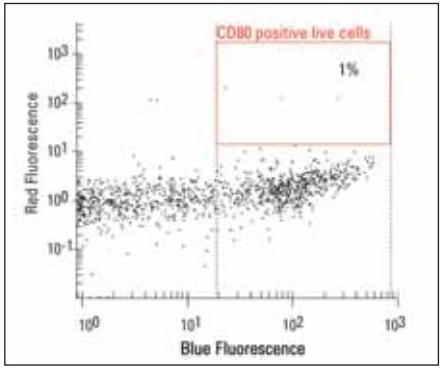
Kit: Cell kit
Assay: On-chip GFP assay
Application: Chinese hamster ovary (CHO-K1) cells were transfected with EGFP DNA by alipofection method. Optimization of transfection conditions were done on one chip. Several DNA:lipofectamine ratios were tried. A ratio of 1:8 gave the best transfection efficiency. All cells were reference stained with a red live dye. On-chip staining was applied, minimizing the staining time, reagent usage and cell consumption.

Application note: 5988-7296EN

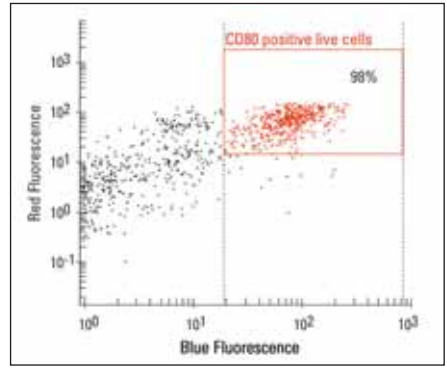
Transfection efficiency monitoring

Verification of stable transfected cell clones by on-chip antibody staining

Hek 293 control cells



CD 80 stable clone

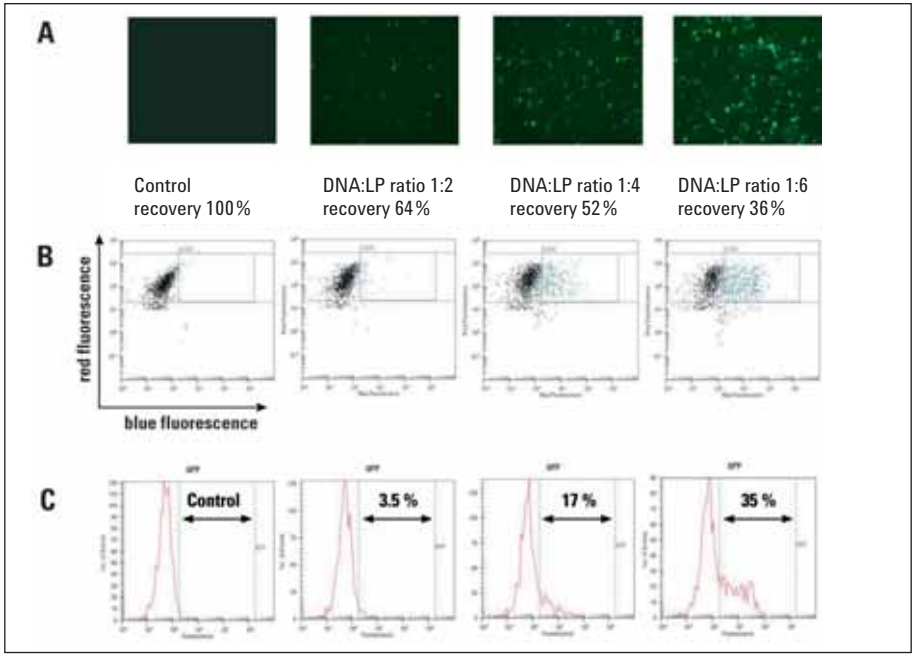


Kit: Cell kit
Assay: On-chip antibody staining assay
Application: Verification of CD80 protein expression in stable transfected Hek 293 cells with the 2100 Bioanalyzer. Control (left dot plot) and CD80 transfected cells (right) are stained on-chip with blue calcein live dye and anti-CD80-CyChrome antibody. Red region marks CD80 protein expressing 293 cells within live cell population – confirming expression in the CD80 stable clone Hek 293 cells.

Application note: 5988-7111EN

Transfection efficiency monitoring

Transfection of primary cells



Kit: Cell kit
Assay: GFP assay
Application: Monitoring the transfection efficiency in primary cells requires low cell consumption, high reproducibility of results, a fast on-chip staining procedure and ease-of-use all provided by the 2100 Bioanalyzer. The transfection efficiency using a GFP-coding plasmid (pEGFP-C2) at varying plasmid:lipofectamine ratios (DNA:LP ratio) obtained with human umbilical vein endothelial cells (HUVEC) is measured in this optimization series. Images from a fluorescence microscope (A) and dot plots (B), as well as histograms (C) of control- and GFP-transfected cells are shown. Using increasing ratios, better transfection efficiency was achieved, whereas the toxicity of LP caused decreased recovery of living cells. Such data facilitates optimizing transfection conditions.

Application note: 5988-8154EN

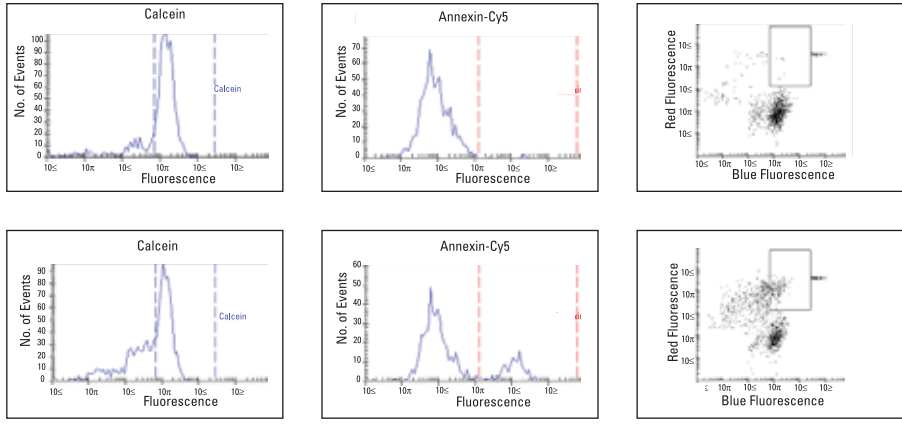
Apoptosis detection

Detection of phosphatidylserine on the cell surface via Annexin V binding

2100 Bioanalyzer histogram: blue channel (Calcein)

2100 Bioanalyzer histogram: red channel (Annexin-Cy5)

2100 Bioanalyzer dot plot: events from both channels



16h treated sample

Subpopulation of all live cells which are apoptotic

Kit: Cell kit

Assay: Apoptosis assay

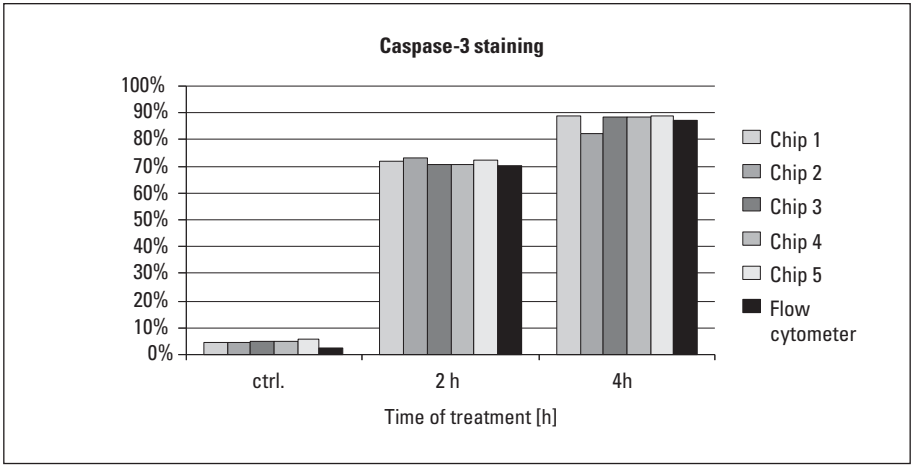
Application: Apoptosis (programmed cell death) in Jurkat cells was induced with camptothecin. Cells treated for 16 hours and untreated cells were stained with calcein and Annexin-Cy5. Annexin-V binds to phosphatidylserine – a membrane lipid which is kept to the inner leaflet of the cell membrane of intact cells. Exposure of phopshatidylserine on the outer leaflet is an early indicator of apoptotic processes. Annexin-V binding is made detectable by Cy5 staining of the Annexin-V via a biotin-streptavidin interaction. Calcein staining of cells is used as a live control to distinguish living and apoptotic cells from dead cells. Calcein enters the cell via the membrane as a non-fluorescent ester. The ester is cleaved inside the cell which results in fluorescence.

The histograms on the left show the number and intensity value of all events which generated a signal in the blue channel, corresponding to calcein-stained cells. The histograms on the right shows all events which generated a signal in the red channel, corresponding to Annexin-V binding to apoptotic cells. While the control shows only low intensity values (background noise), the treated sample shows high intensity values (within the red markers) corresponding to apoptotic cells. The dot plot of the treated sample nicely shows the subpopulation of all live cells which are apoptotic.

Application note: 5988-4319EN

Apoptosis detection

Intracellular Caspase-3 antibody staining assay



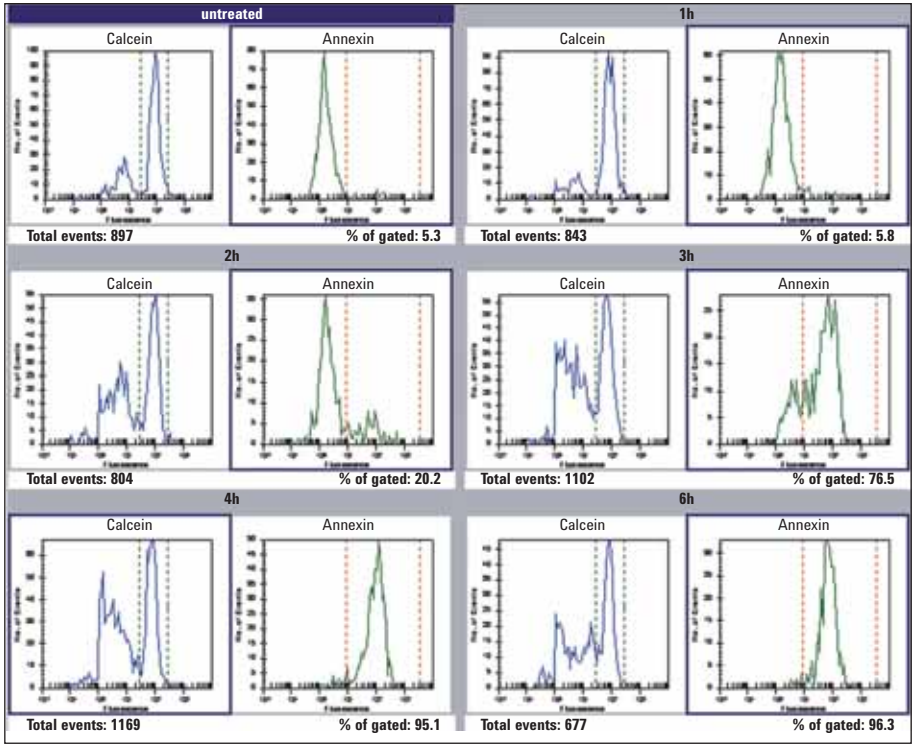
Kit: Cell kit
Assay: Generic assay
Application: Induction of apoptosis in Jurkat cells was done with anti-FAS antibody treatment. Intracellular staining with specific antibodies against 'active' Caspase-3 were performed. Reference staining was done with SYTO16 DNA dye. Good chip to chip reproducibility and good comparison to conventional flow cytometer results were obtained.

Application note: 5988-4319EN

Apoptosis detection

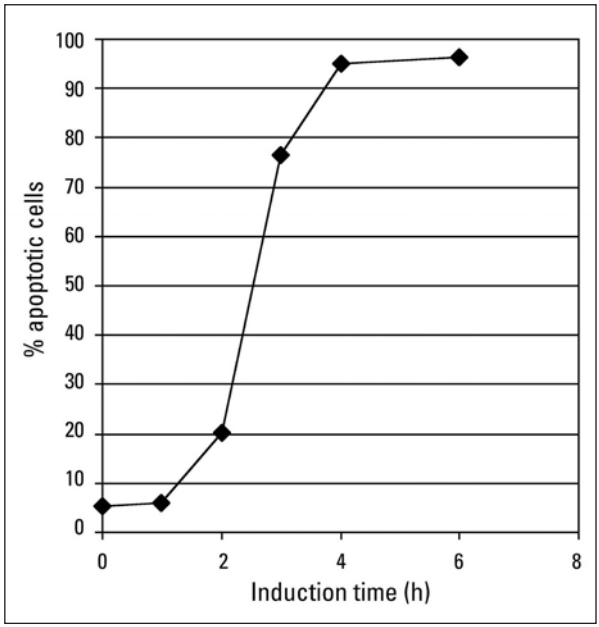
Fast Annexin protocol for time course of apoptosis induction via anti-FAS antibody

A



Kit: Cell kit
Assay: Apoptosis assay
Application: Apoptosis (programmed cell death) in Jurkat cells was induced with anti-FAS antibody. Cells treated for 0,1,2,3,4 and 6 hours were stained with calcein and Annexin-Cy5. Annexin-V binds to phosphatidylserine – a membrane lipid which is kept to the inner leaflet of the cell membrane of intact cells. Exposure of phopshatidylserine on the outer leaflet is an early indicator of apoptotic processes. Annexin V binding is detectable by Cy5 staining of the Annexin-V via a biotin-streptavidin interaction. Calcein staining of cells is used as a live control to distinguish living and apoptotic cells from dead cells. Calcein enters the cell via the membrane as non-fluorescent ester. The ester is cleaved inside the cell which results in fluorescence and indicates apoptosis.

B

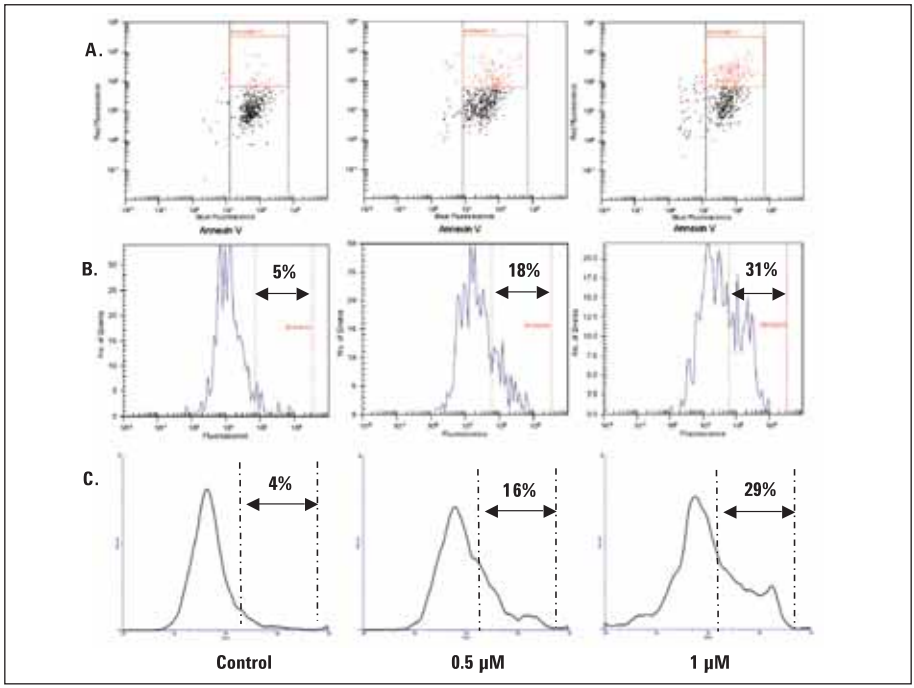


The histograms on page 24 (A) show the number and intensity value of all events which generated a signal in the blue channel, corresponding to calcein-stained cells. The histograms on the right show all events which generated a signal in the red channel, corresponding to Annexin-V binding to apoptotic cells. While the control shows only low intensity values (background noise), the treated sample shows high intensity values (within the red markers) corresponding to apoptotic cells.
(B) Time course of the induction of apoptosis by anti-FAS antibody in Jurkat cells. Apoptosis is detectable in a significant amount of cells after 2 hours. Following a treatment of 4 hours, approximately 95 % of the cells are apoptotic.

Application note: 5988-7297EN

Apoptosis detection

Apoptosis detection in primary cells



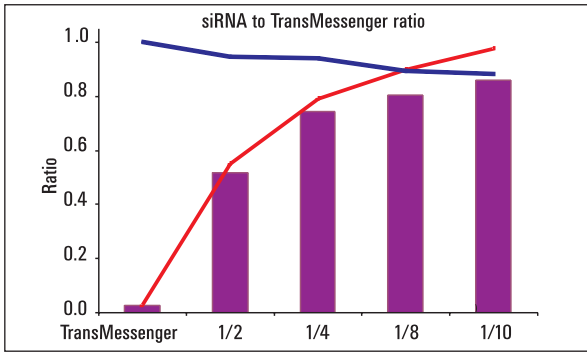
Kit: Cell kit
Assay: On-chip antibody staining assay
Application: The 2100 Bioanalyzer has been used to study induced apoptosis by monitoring annexin V-binding in primary human endothelial cells (HUVEC, not shown) and human dermal fibroblasts (NHDF, shown). A simple and fast assay protocol was used on cells left untreated or treated for 5 hours with different concentrations of staurosporine, which induces apoptosis. See row A for dot blots and B for histograms at different concentrations. Evaluation of the same samples on a conventional flow cytometer (row C) yielded similar results.

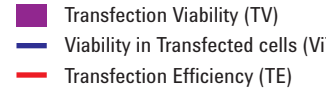
Application note: 5989-2934EN

Gene silencing in cell culture

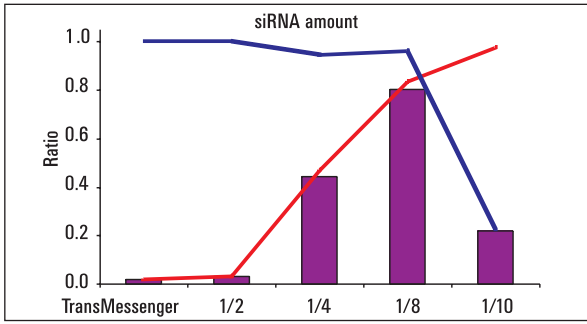
siRNA transfection optimization

A





B



Kit: Cell kit

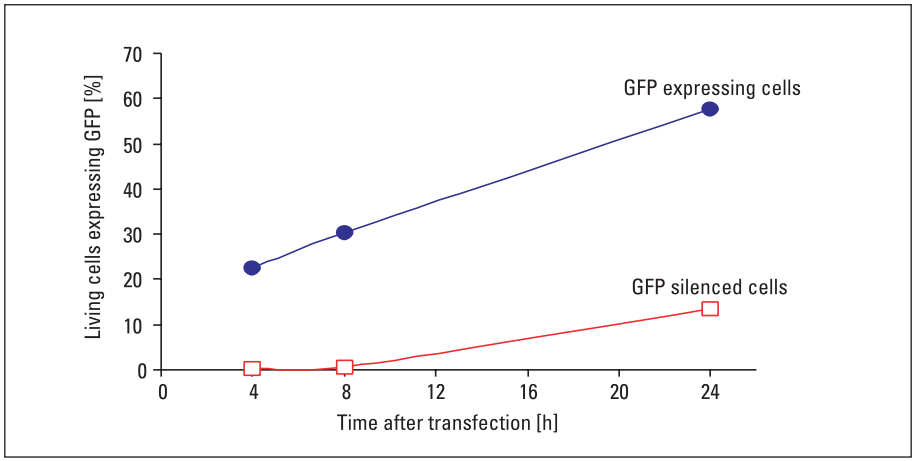
Assay: On-chip antibody staining assay

Application: In gene silencing experiments (HeLa cells) we found that increasing amounts of transfection reagent (TransMessenger™) to a constant amount of siRNA leads to a plateau of transfection viability (panel A). Transfection viability reflects the product of the viability of the transfected cells and the transfection efficiency. With a constant siRNA/transfection reagent ratio of 1:4 and increasing total amounts of introduced siRNA (panel B) the viability of transfected cells decreases at a certain point although the transfection efficiency increases. Thus, there are experimental conditions where the number of living and transfected cells are at a maximum. The 2100 Bioanalyzer features on-chip staining and leads to excellent results with a minimal consumption of cells and reagents.

Application note: 5988-9872EN

Gene silencing in cell culture

Monitoring of gene silencing experiments

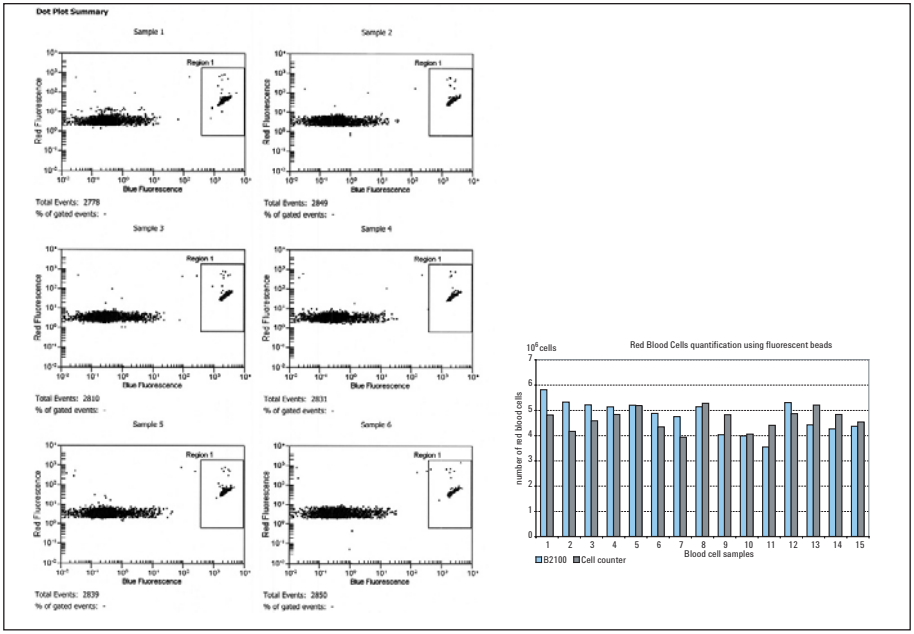


Kit: Cell kit
Assay: GFP Assay
Application: After co-transfection of a GFP plasmid and Cy5-labeled siRNA (GFP-specific), GFP expression and viability of cells were detected. The course of GFP expression in control (GFP only) and siRNA/GFP transfected cells was measured on the 2100 Bioanalyzer. Accurate results were obtained fast and in an automated manner. They easily allow the efficiency and reliability of a given protocol and transfection reagents to be judged. Thus, such an experiment provides efficient monitoring and optimization of any gene silencing experiment.

Application note: 5989-0103EN

Cells – others

Identification and counting of blood cells in whole blood samples



Kit: Cell kit
Assay: Generic assay
Application: Nucleic acid dye and antibody staining was used in accordance with the dual-fluorescence detection system of the 2100 Bioanalyzer to identify several blood cell populations and count blood cells from whole blood samples without a washing step. The red blood cells (RBC) are stained with an anti-CD235a monoclonal antibody conjugated with the fluorochrome.

A) On-chip antibody staining results as dot plots for 6 samples samples including a region with spiked-in beads for quantitation.
B) Quantitative determination of RBC counts with the 2100 Bioanalyzer and an automatic blood cell counter. The microvolumes of samples and reagents, the low number of cells required for the analysis as well as the easy use of the 2100 Bioanalyzer are the specific advantages of this microfluidic chip-based technology in comparison with the cell counter.

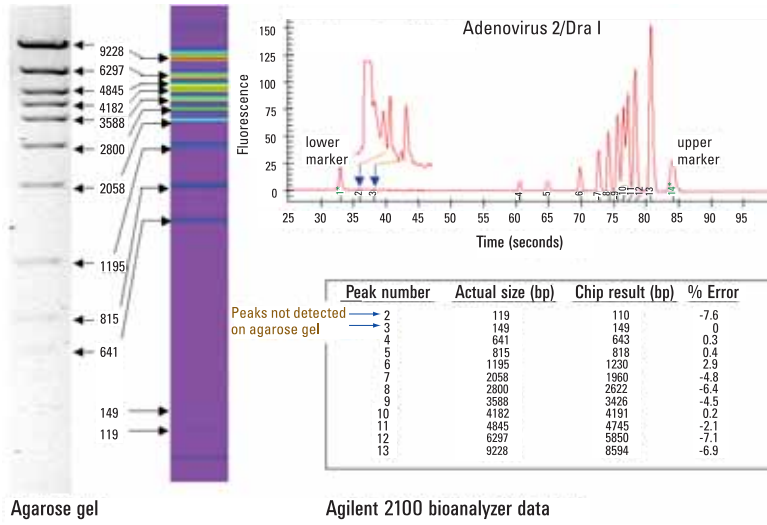
Application note: 5989-7171EN

II. DNA analysis

	Agriculture/Food	Drug Discovery/Drug Development	Drug Manufacturing QA/QC	Genomics	Pharmaceuticals	Proteomics	Forensics/Homeland Security
Restriction digest analysis							
Sizing range exemplified by the separation of Adenovirus 2/Dra I				●			
Detection of single base mutations (I)				●			●
Detection of single base mutations (II)				●			●
PCR product analysis							
Separation of 3 different mixtures of PCR products				●			●
Optimize QPCR assay design	●			●			●
DNA sample quality from automated PCR purification				●			
Determination of PCR product impurity				●			●
Multiplex PCR analysis of bacteria in chicken	●			●			●
Multiplex PCR with 19 products	●			●			●
Gene expression analysis							
mRNA expression study by comparative multiplex PCR	●	●		●			
Standardized end-point RT-PCR	●	●		●			
Co-amplification of GAPDH and hsp72				●			
Co-amplification of GAPDH and hsp72 – response curves				●			
Competitive PCR	●			●			●
Food analysis							
Estimation of non-basmati rice amounts in basmati rice products	●			●			
Development of meat specific assays (I)	●			●			
Development of meat specific assays (II)	●			●			
Fish species identification by RFLP	●			●			
GMO detection							
Development of a multiplex assay for soya	●			●			
DNA stability during food processing	●			●			
GMO detection by nested multiplex PCR	●			●			
Oncology							
Tumor cell detection from carcinoma patient blood		●		●			
SNP analysis in cancer related P16 gene		●		●			
K-ras gene SNP detection		●		●			
METH-2 downregulation in lung carcinomas		●		●			
Label-free analysis of microsatellite instability in carcinoma		●		●			
Clinical research							
Detection of a point mutation in the prothrombin gene with PCR-RFLP		●		●			
Genotyping of H. pylori	●	●		●			●
Duplications and deletions in genomic DNA		●		●			
Forensic testing							
Optimization of PCR on mtDNA				●			●
Pitfalls in mtDNA sequencing				●			●
Next-generation sequencing							
DNA library quantity and quality		●		●			
DNA library QC in target enrichment and next-gen sequencing workflows		●		●			
Sizing and quantitation of DNA libraries and fragmented DNA		●		●			

Restriction digest analysis

Sizing range exemplified by the separation of Adenovirus 2/Dra I



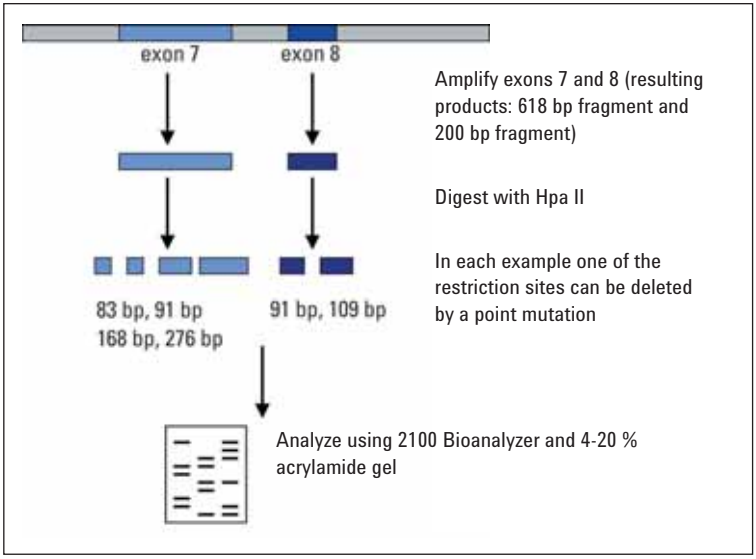
Peak number	Actual size (bp)	Chip result (bp)	% Error
2	119	110	-7.6
3	149	149	0
4	641	643	0.3
5	815	818	0.4
6	1195	1230	2.9
7	2058	1960	-4.8
8	2800	2622	-6.4
9	3588	3426	-4.5
10	4182	4191	0.2
11	4845	4745	-2.1
12	6297	5850	-7.1
13	9228	8594	-6.9

Kit: DNA 12000 kit
Assay: DNA 12000 assay
Application: Restriction digest analysis of Adenovirus 2/Dra I. For restriction fragment analysis the large linear dynamic range of the lab-on-a-chip approach is very advantageous. Analyzing samples with large and short fragments on slab gels can be difficult because of bands running off the gel and insufficient staining (or over-staining) of bands.

Application note: 5968-7501EN

Restriction digest analysis

Detection of single base mutations (I)

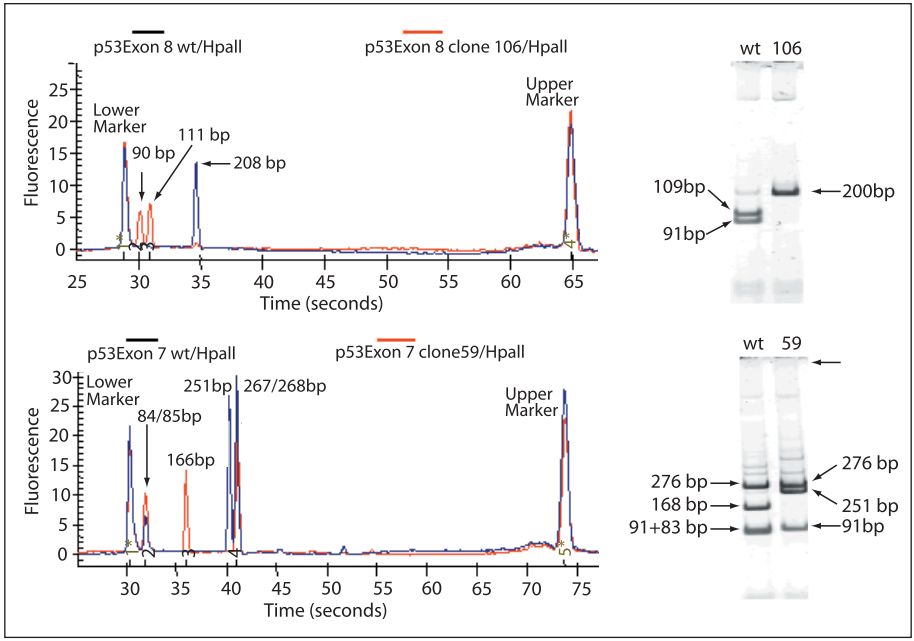


Kit: DNA 7500 kit
Assay: DNA 7500 assay
Application: Mutation detection by RFLP highlights the use of the 2100 Bioanalyzer. Two different regions of the p53 gene were amplified with specific primers and digested with Hpa II, which cuts in a location that is prone to mutations. In the presence of a point mutation, the enzyme Hpa II does not cleave the DNA, leaving larger fragments that can be revealed by gel electrophoresis or by analysis with the DNA 7500 kit (see next page).

Data not published

Restriction digest analysis

Detection of single base mutations (II)

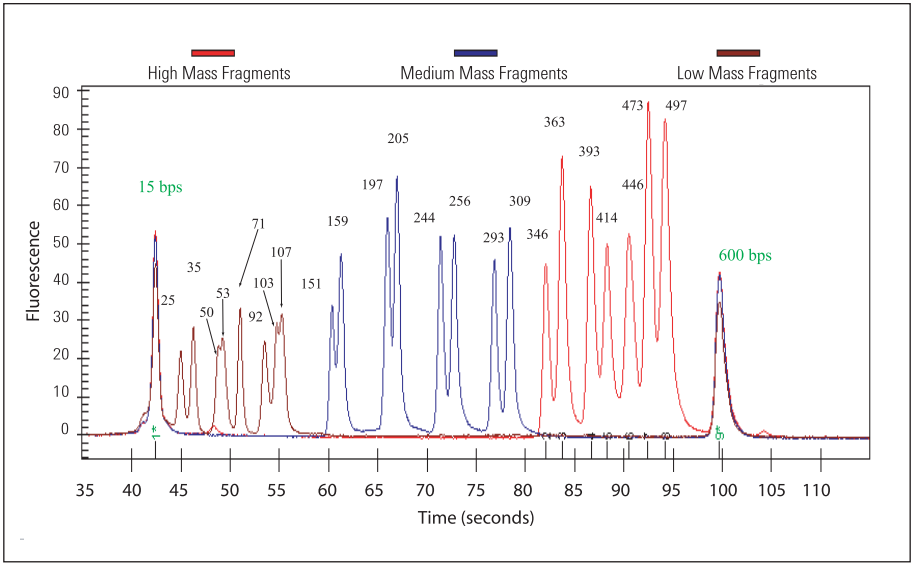


Kit: DNA 7500 kit
Assay: DNA 7500 assay
Application: Analysis on the chip showed an identical pattern of digest fragments as seen on the slab gel for the wildtype and Exon 7 & 8 PCR products. Comparison of the calculated sizes of the bands shows 1-2 % variance with the assay, which allows fast and accurate detection of point mutations.

Application note: 5968-7496EN

PCR product analysis

Separation of 3 different mixtures of PCR products



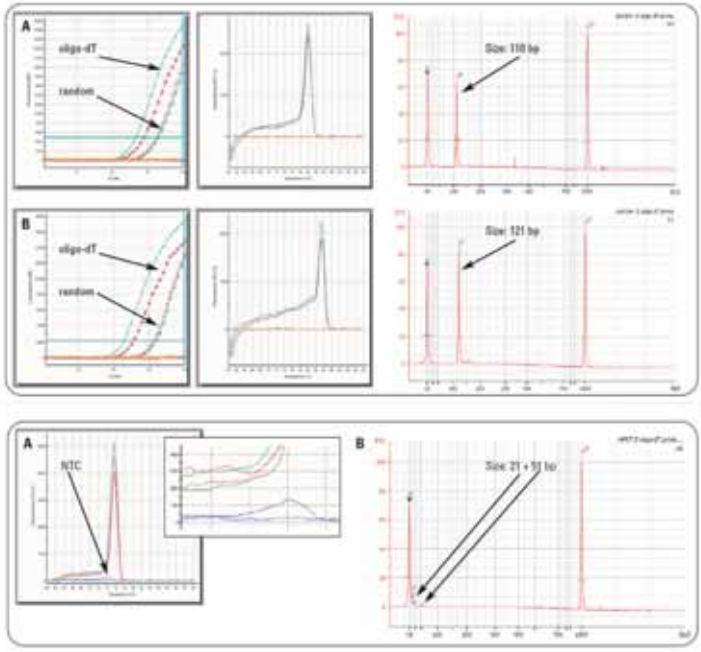
Kit: DNA 500 kit*
Assay: DNA 500 assay*
Application: Overlay of three different electropherograms, which are mixtures of PCR samples ranging from 25 to 500 base pairs in size. The two closest eluting bands (50 bp and 53 bp) are partially separated and identified by the software as two separate peaks. The DNA 500* assay achieves a resolution of five base pairs from 25 to 100 base pairs and a 5 % resolution from 100 to 500 base pairs where the sizing error is less than 10% over the entire size range.

Application note: 5988-3041EN

* replaced with DNA 1000 kit and assay

PCR product analysis

Optimize QPCR assay design

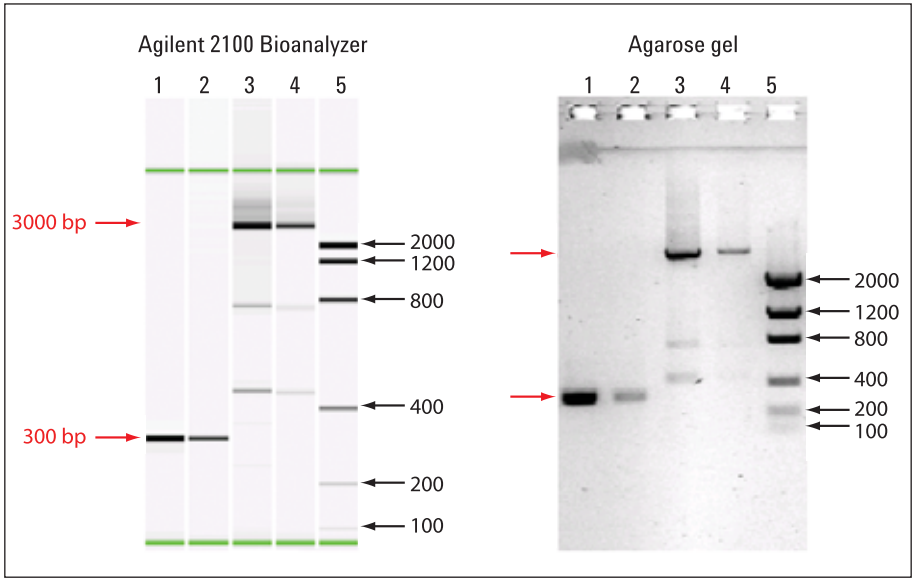


Kit: DNA 1000 kit
Assay: DNA 1000 assay
Application: To determine the best possible RT priming strategy, QPCR and 2100 Bioanalyzer tests were conducted for three genes (GAPDH, HPRT1 and YWHAZ) by using high quality RNA (RIN = 10) as template. The RNA was reverse transcribed with either oligo-dT or random priming (A: 5′-assay, B: 3′-assay for GAPDH). To assess size and purity of QPCR amplicons, 1 µL of the QPCR reactions was analyzed with the DNA 1000 assay. In addition, No-template-controls (NTC) were used to assess contamination and potential primer dimer formation, since already a small amount of contaminating template can lead to amplification. For the HPRT1 5′ oligo-dT assay one of the NTC was positive, showing a peak in the melt curve at a similar melting temperature compared to the positive control (A). To verify that no contamination of the well has occurred, the NTC was analyzed using the DNA 1000 assay on the 2100 Bioanalyzer (B). Two minor peaks could be detected at 21 and 51 bp which are most probably related to primer and primer dimers. This highlights the high information content obtained by the 2100 Bioanalyzer and the poor discrimination capabilities of a SYBR Green based melt curve.

Application note: 5989-7730EN

PCR product analysis

Determination of PCR product impurity

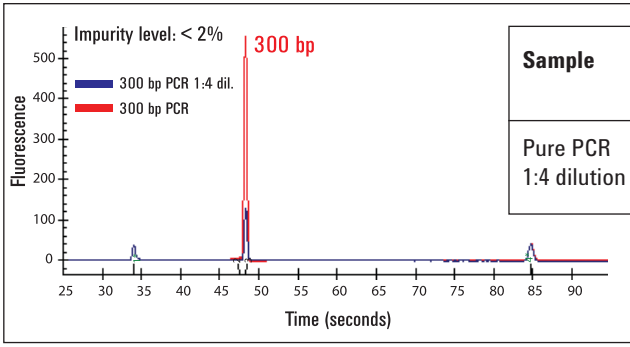


Kit: DNA 7500 kit
Assay: DNA 7500 assay
Application: Comparison between the analysis of two PCR reactions (300 and 3000 bp products) using the DNA 7500 kit vs. an agarose gel. Two different concentrations are shown side by side for each PCR reaction (undiluted and 1:4 dilution). The 2100 Bioanalyzer shows superior performance in locating impurities over a broader concentration range than the gel. The 300 bp fragment appears to be uncontaminated in both the gel and on the 2100 Bioanalyzer.
The 3000 bp fragment shows few impurities on the gel, which become invisible at the 1:4 dilution. These impurities can easily be detected with the 2100 Bioanalyzer.

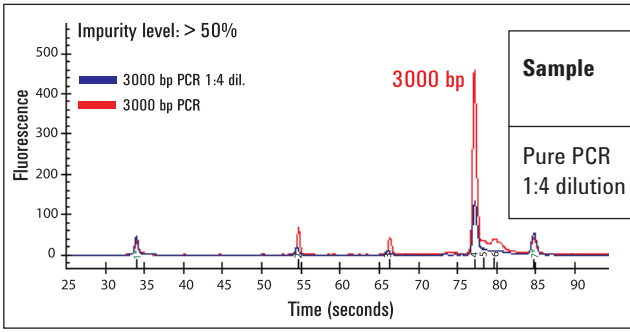
Application note: 5968-7496EN

PCR product analysis

Determination of PCR product impurity



Sample	c (DNA) All peaks	Main peak (300 bp)
Pure PCR	41.4 ng/µL	40.7 ng/µL
1:4 dilution	9.6 ng/µL	9.6 ng/µL



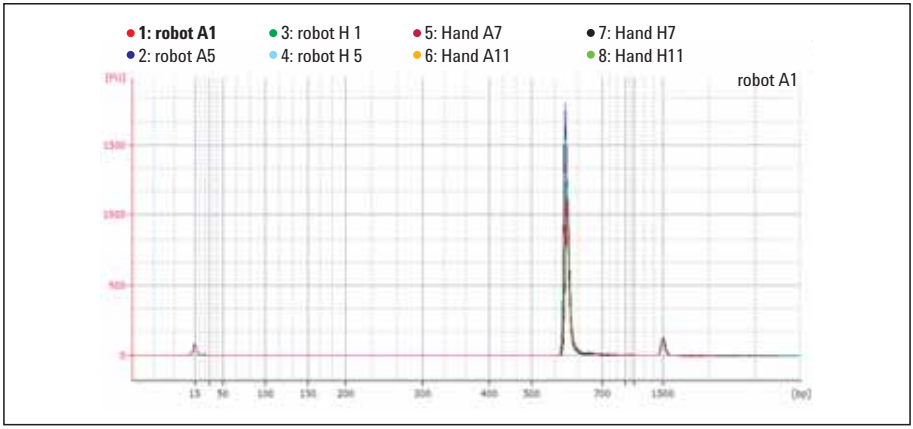
Sample	c (DNA) All peaks	Main peak (3000 bp)
Pure PCR	61.9 ng/µL	40.7 ng/µL
1:4 dilution	14.8 ng/µL	9.8 ng/µL

Kit: DNA 7500 kit
Assay: DNA 7500 assay
Application: The quantitative data generated by the 2100 Bioanalyzer indicate the amount of impurity or non-specific products in the PCR reactions from the previous page. Even in the 300 bp fragment a small impurity can be detected, while the 3000 bp fragment shows more than 50 % impurities.

Application note: 5968-7496EN

PCR product analysis

DNA sample quality from automated PCR purification

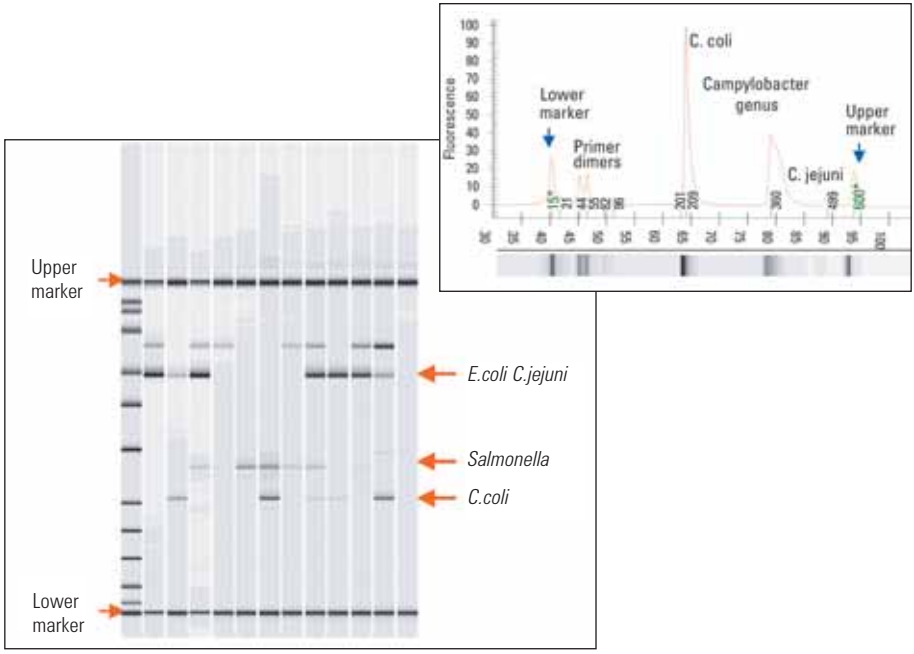


Kit: DNA 1000 kit
Assay: DNA 1000 assay
Application: A protocol for automated PCR purification in a 96-well format employing the Bravo Automated Liquid Handling Platform, Automated Centrifuge and StrataPrep 96 PCR Purification kit from Agilent was compared to a manual method. The DNA recovery quality from four wells from different portions of the plate was assessed with the 2100 Bioanalyzer and the DNA 1000 kit. The figure shows overlaid samples from both manually-purified and robot-purified samples, demonstrating identical sizing and purity. The single peak at just over 600 bp is correctly sized within 1 % of the predicted PCR product size. There are no contaminating peaks or primers formed during PCR of Lambda DNA. The smaller peaks at 15 and 1500 bp are DNA markers.

Article: 5990-3948EN

PCR product analysis

Multiplex PCR analysis of bacteria in chicken



Data kindly provided by GenPoint, NL

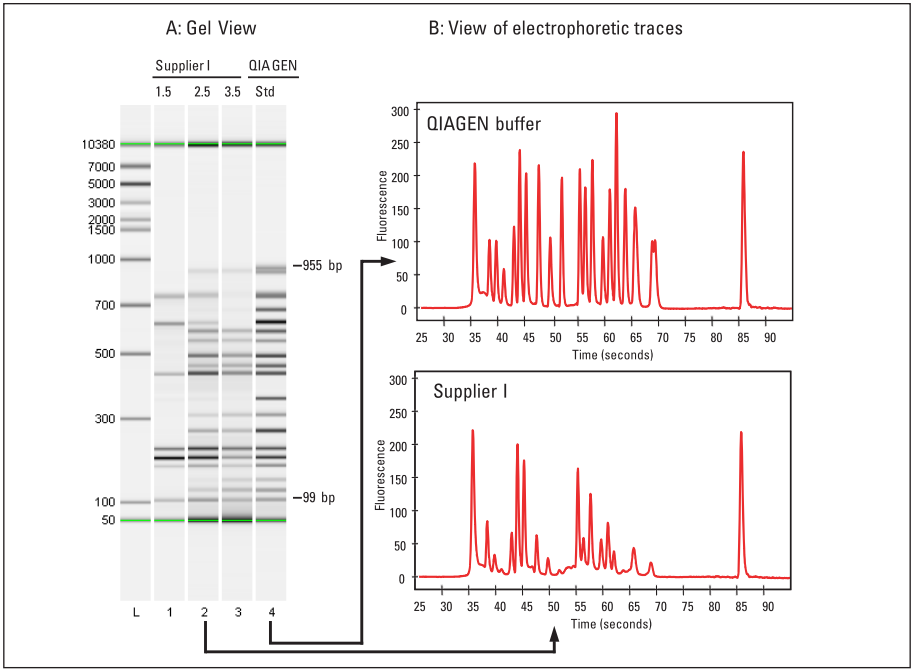
Kit: DNA 500 kit*
Assay: DNA 500 assay*
Application: Multiplex PCR with four primer pairs, each one specific for a certain DNA sequence from one of the 4 bacteria to be tested for. Total DNA was extracted from chicken and subjected to PCR. The gel-like image shows traces from different chicken samples with bands showing up when an amplicon could be detected. The electropherogram is one example where bacterial DNA from two species of the *Campylobacter* genus could be detected.

Data not published

* replaced with DNA 1000 kit and assay

PCR product analysis

Multiplex PCR with 19 products



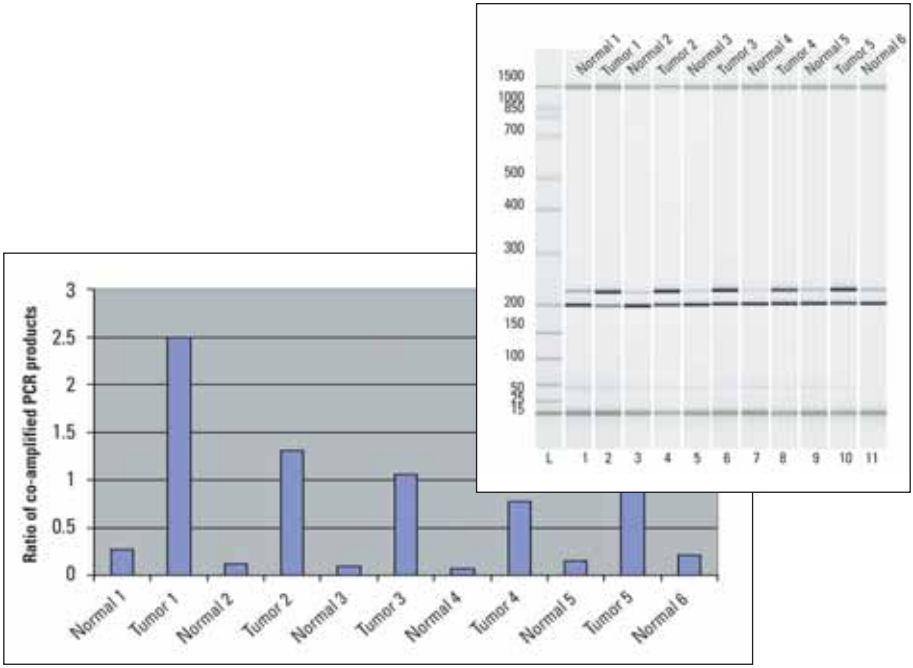
Data kindly provided by Qiagen, Germany

Kit: DNA 7500 kit
Assay: DNA 7500 assay
Application: Many molecular applications include PCR multiplexing as shown above with a PCR that yields 19 products. Applications are genotyping of transgenic organisms, detection of pathogens or GMs and microsatellite genotyping (e.g. short tandem repeat (STR) and variable number tandem repeat (VNTR) analyses). The sample shows optimization of PCR conditions (Mg^{2+} concentration) performed to ensure annealing of the multiple primers under identical conditions. Visualization and evaluation of the results can be performed efficiently with the 2100 Bioanalyzer because of the high resolution, the accurate sizing, quantitation and extended linear range.

Application note: 5988-9342EN

Gene expression analysis

mRNA expression study by comparative multiplex PCR



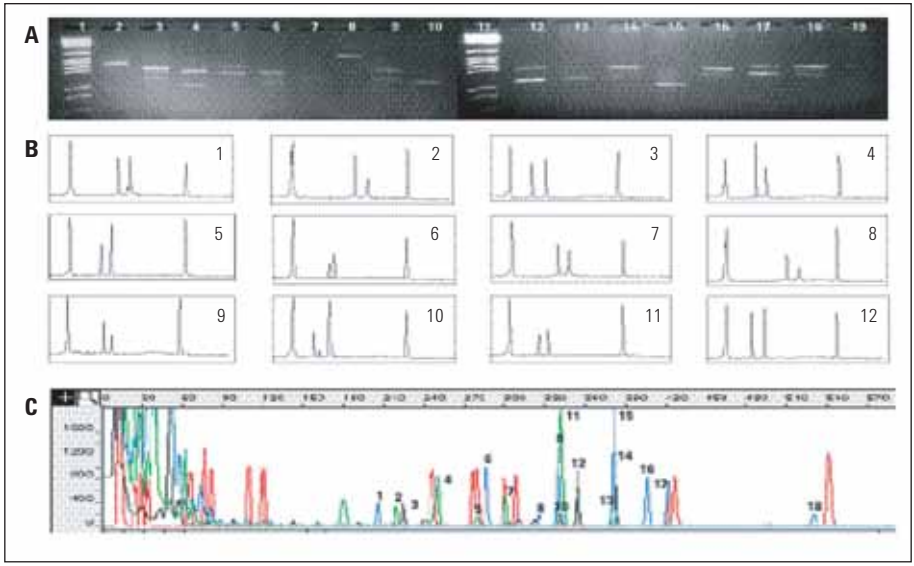
Data kindly provided by the Roy Castle Centre

Kit: DNA 1000 kit
Assay: DNA 1000 assay
Application: Two genes were co-amplified in this study. A tumor specific gene (upper band) along with a housekeeping gene (lower band). The upregulation of the tumor gene is visualized via analysis on the 2100 Bioanalyzer. Building the ratio of the concentration values obtained from the 2100 Bioanalyzer, numerical values are obtained that are normalized with regard to the RT-PCR amplification efficiency. This way tumor tissue can be distinguished from normal tissue more unambiguously.

Data not published

Gene expression analysis

Standardized end-point RT-PCR



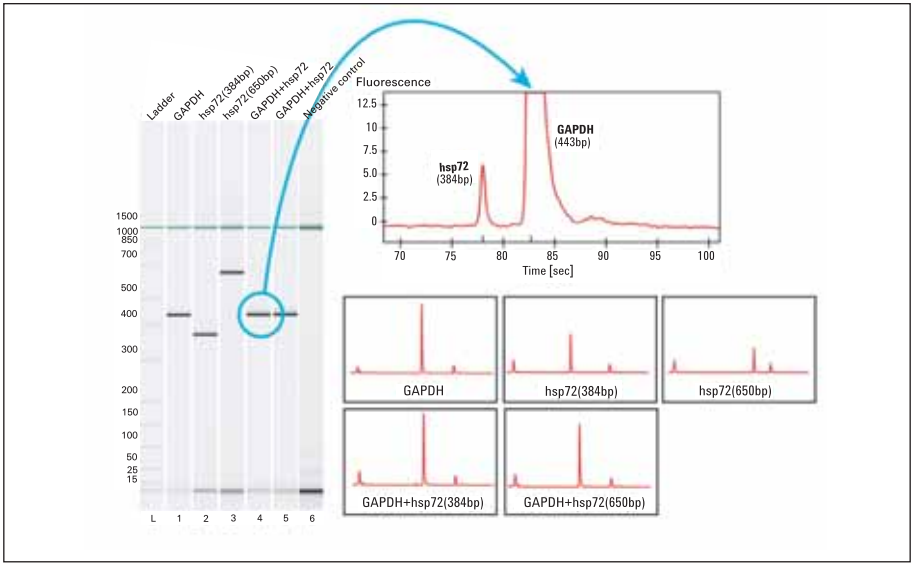
Data kindly provided by the Medical College of Ohio

Kit: DNA 7500 kit
Assay: DNA 7500 assay
Application: Complementary DNA from bronchial epithelial cells (BEC) was analyzed by a Standardized RT-PCR (StaRT) for the expression of 15 different genes. This analysis can be performed at the end-point of PCR without the need for real-time measurement at each cycle of PCR. Three methods for evaluation of representative results were compared (see above). The coefficient of variance (CV) from at least 3 measurements was calculated. The direct comparison of the reproducibility for agarose gel analysis (A, CV = 0.50) and the ABI Prism310 Genetic Analyzer (C, CV = 0.39) with the 2100 Bioanalyzer (B, CV = 0.29) reveals that the 2100 Bioanalyzer is superior. It is a reliable and valuable tool in quantitative gene expression analysis.

Application note: 5988-3674 EN

Gene expression analysis

Co-amplification of GAPDH and hsp72



Data kindly provided by Dr. Eric Gottwald, Forschungszentrum Karlsruhe, Germany

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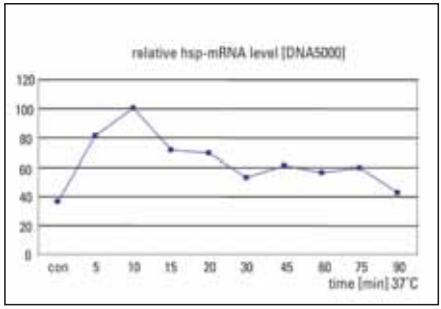
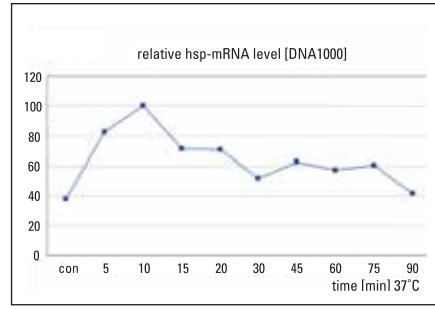
Kit: DNA 1000 kit
Assay: DNA 1000 assay
Application: Gel-like image and electropherograms showing the results of separate amplifications and co-amplifications of GAPDH and hsp72 in unstimulated HepG2 cells. Primers for GAPDH yield a PCR product of 443 bp (lane 1), primers for hsp72 yield PCR products of 384 and 650 bp (lane 2 and 3). Lane 4 and 5 show the results of the co-amplification reactions. Due to the competitiveness of the reaction, very little hsp72 products could be detected in lane 4 (insert) and no product was detected in lane 5 (lane 6 = negative control). The broad linear dynamic range of the analysis allows detection of weak bands next to strong bands and helped in the determination of gene expression in this case.

Article: 5988-4556EN

Gene expression analysis

Co-amplification of GAPDH and hsp72 – response curves





Data kindly provided by Dr. Eric Gottwald, Forschungszentrum Karlsruhe, Germany

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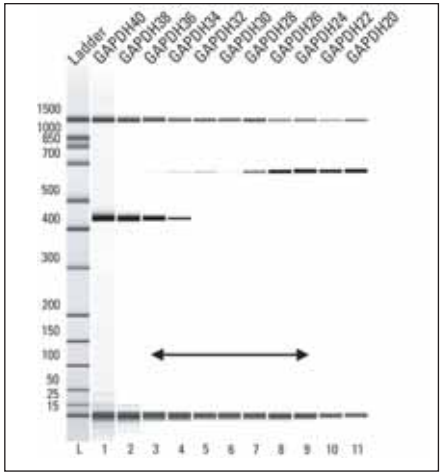
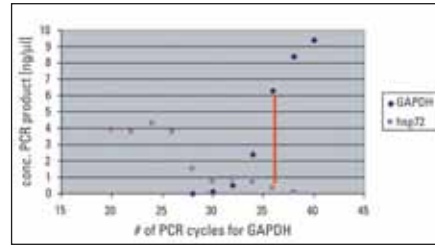
Kit: DNA 1000 kit
Assay: DNA 1000 assay
Application: The optimized PCR conditions were used to monitor the response of a stimulus to hsp. Gene expression was monitored by comparing the RT-PCR amplification of a housekeeping gene with the co-amplification of hsp. In the current case, the highest gene expression was measured after about 10 minutes. As a comparison, the same set of samples was analyzed using the DNA 500 kit*. Virtually identical results are obtained with both kits, demonstrating thatlab-on-a-chip technology can serve as a standardized approach to gel electrophoresis.

Article: 5988-4556EN

Gene expression analysis

Competitive PCR





Data kindly provided by Dr. Eric Gottwald, Forschungszentrum Karlsruhe, Germany

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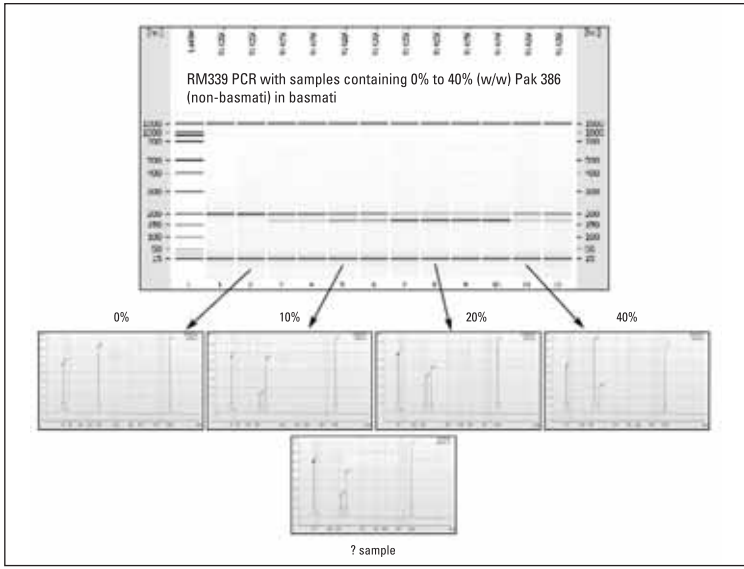
Kit: DNA 1000 kit
Assay: DNA 1000 assay
Application: Two genes were reverse transcribed and co-amplified in one reaction tube. The PCR products were analyzed using the DNA 1000 kit. Primers for hsp72 were present from the beginning of the PCR reactions, while primers for GAPDH were added after various cycle numbers ranging from 20 to 40 cycles (primer dropping method). This allowed optimization of this competitive PCR reaction. The left graph displays the dynamic range (arrow) in the gel like view, whereas the right graph indicates conditions with greatest sensitivity (red line).

Article: 5988-4556EN

Food analysis

Estimation of non-basmati rice amounts in basmati rice products

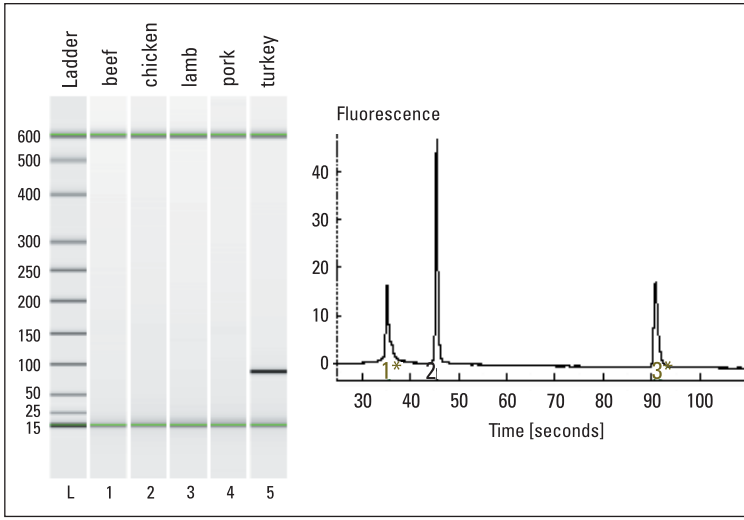


Kit: DNA 1000 kit
Assay: DNA 1000 assay
Application: Ensuring integrity of raw food materials, ingredients, and products is both a product quality and regulatory compliance concern. Food ingredient suppliers, manufacturers, and retailers can suffer economic and legal damages if proven to be supplying materials or products that are incorrectly labeled due to substitution or contamination. For example, EU Commission 1549/04 grants lower import tax on nine basmati rice varieties. Furthermore, the level of non-basmati rice in a basmati rice product must not exceed 7 %. Therefore it is required to identify basmati rice varieties and determine the level of non-basmati rice in basmati rice products. The 2100 Bioanalyzer and the DNA 1000 kit can be used as a quick and cost-effective analytical method to differentiate approved and non-approved rice varieties using three primer sets and to estimate the level of non-basmati rice using reference rice admixtures. Four reference samples with 0 to 40 % non-basmati rice content were used to estimate the non-basmati amount in an unknown sample to be 10-20 %.

Application note: 5989-6836EN

Food analysis

Development of meat specific assays (I)



Data kindly provided by CCFRA, UK

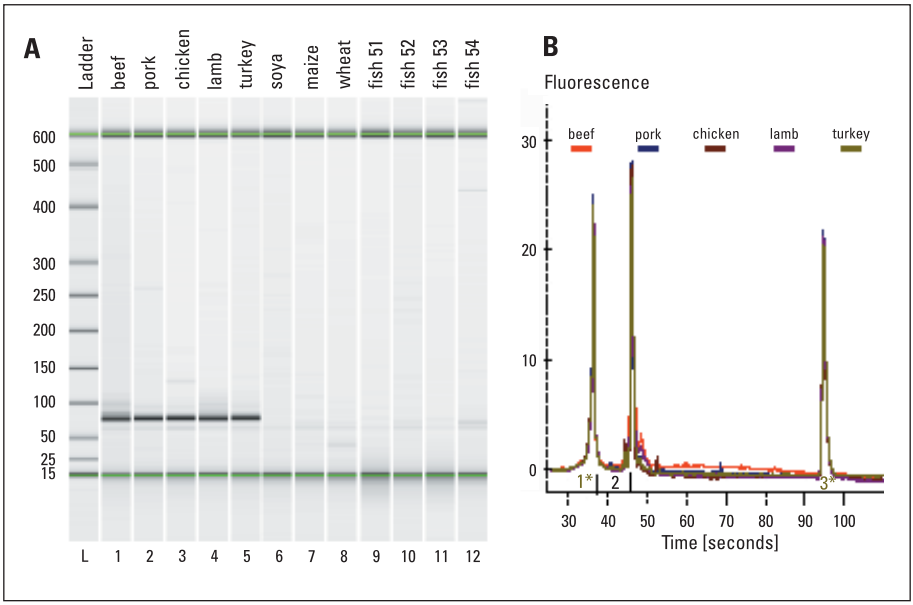
Kit: DNA 500 kit*
Assay: DNA 500 assay*
Application: For detection of individual species in processed food, PCR assays with specific sets of primers can be developed. Example: turkey specific primers do not amplify any other meat species, including beef, chicken, lamb, or pork (see lane 5 and respective electropherogram).

Application note: 5988-4069EN

* replaced with DNA 1000 kit and assay

Food analysis

Development of meat specific assays (II)



Data kindly provided by CCFRA, UK

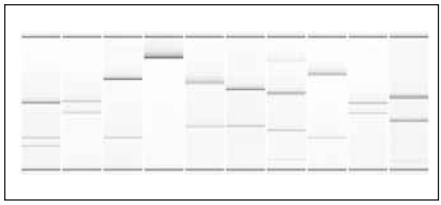
Kit: DNA 500 kit*
Assay: DNA 500 assay*
Application: For detection of individual component types in processed food, PCR assays with specific sets of primers can be developed. Example: Primers that amplify any type of meat, but do not amplify other food constituents, including soya, maize, wheat or fish.

Application note: 5988-4069EN

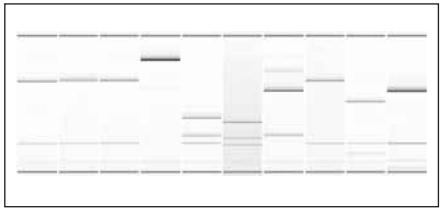
* replaced with DNA 1000 kit and assay

Food analysis

Fish species identification by RFLP



Restriction enzyme: DdeI



Restriction enzyme: HaeIII

Restriction enzyme: NlaIII

Common name (UK)	Latin name
Atlantic Cod	Gadus morhua
Pacific Cod	Gadus macrocephalus
Coley (Saithe)	Pollachius virens
Haddock	Melanogrammus aeglefinus
European Hake	Merluccius merluccius
South African Hake	Merluccius paradoxus
European Plaice	Pleuronectes platessa
Whiting	Merlangus merlangus
Alaskan (Walleye) Pollock	Theragra chalcogramma
Hoki	Macruronus novaezelandiae
Atlantic Salmon	Salmo salar
Red / Sockeye Salmon	Oncorhynchus nerka
Pink / Humpback Salmon	Oncorhynchus gorbuscha
Chinook Salmon	Oncorhynchus tschawytscha
Coho / Silver Salmon	Oncorhynchus kisutch
Keta / Chum Salmon	Oncorhynchus keta
Cut-throat Trout	Oncorhynchus clarki clarki
Dolly Varden	Salvelinus malma malma
Cherry Salmon	Oncorhynchus masou masou

Data kindly provided by CCFRA, UK

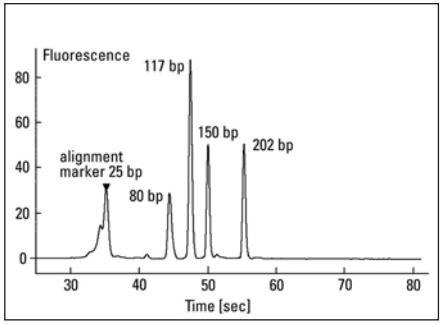
Kit: DNA 500 kit*
Assay: DNA 500 assay*
Application: Identification of white fish and salmon species in the processed state presents a challenge. However, evaluation of PCR-RFLP profiles (PCR-restriction fragment length polymorphism) of a 464 bp region from the cytochrom b gene cut separately with three restriction enzymes facilitated the differentiation of 19 commercially important species. Analysis of the restriction digests was performed with the 2100 Bioanalyzer. This approach was successfully tested in an interlaboratory study.

Application note: 5989-2982EN

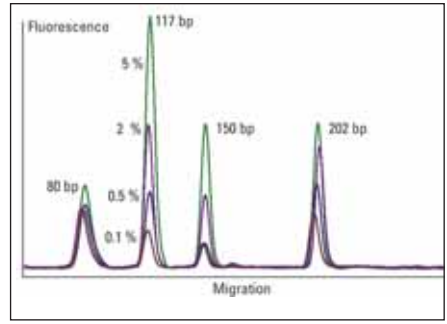
GMO detection

Development of a multiplex assay for soya

A



B



Data kindly provided by CCFRA, UK

Kit: DNA 500 kit*
Assay: DNA 500 assay*
Application: Multiplex assay for genetically modified (GM) soya. The aim was to develop a model assay that could be used to assess the quality of DNA extracted from heat-processed soya flour samples, in particular, to investigate differences in PCR amplification between small DNA targets. A single multiplex PCR assay was developed that enabled three GM soya targets and one control to be analyzed in a single reaction mix. Primer concentration was optimized in order to obtain four PCR products resolved by gel electrophoresis which corresponded in size to the soya lectin gene target of 80 bp, and the EPSPS (5-enolpyruvyl-shikamate-3-phosphate synthase) gene targets of 117 bp, 150 bp and 202 bp respectively. These latter targets are only found in Roundup Ready GM soya. Figure A: Peaks produced by the four PCR products when analyzed with the 2100 Bioanalyzer and DNA 500 kit*. Figure B: Analysis of certified reference materials containing known amounts of GM soya.

Application note: 5988-4070EN

* replaced with DNA 1000 kit and assay

GMO detection

DNA stability during food processing

Time at 100°C and pH 3.3 (min)	Amount of PCR product*			
	80 bp	118 bp	150 bp	202 bp
0	100	100	100	100
3	74	77	73	67
6	57	58	21	6
9	36	23	24	15
12	67	33	47	21
15	48	27	16	0
18	0	0	0	0
21	0	0	0	0

* % product determined relative to the amount at 0 minutes

Data kindly provided by CCFRA, UK

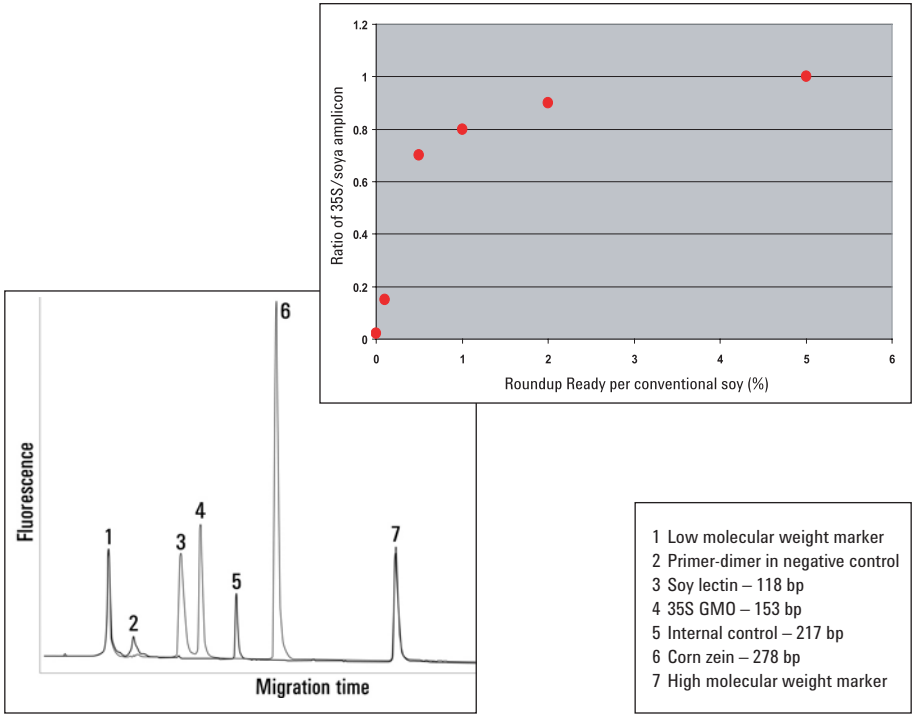
Kit: DNA 500 kit*
Assay: DNA 500 assay*
Application: The multiplex PCR assay was applied to soya flour samples containing approx. 1.3 % GM soya and boiled at either pH 3.3, 4.3 or 6.7 for up to 21 minutes. For accurate determination of the quantity of each PCR product, the samples were analyzed with the DNA 500 kit*. The concentration of each PCR product was calculated using the 2100 Bioanalyzer software. At pH 3.3 where an effect of heating time was observed, the amount of each PCR product at each time point was compared to the amount of each product at 0 minutes (Table 2). At pH 3.3, the relative amount of the 80 bp product was reduced to 48 % after 15 minutes and no product was detected at 18 or 21 minutes. After 15 minutes, the relative amounts of products of 118 bp and 150 bp were reduced to 27 % and 16 % respectively and the 202 bp product was not detected. None of the products were detected after 18 or 21 minutes.

Application note: 5988-4070EN

* replaced with DNA 1000 kit and assay

GMO detection

GMO detection by nested multiplex PCR



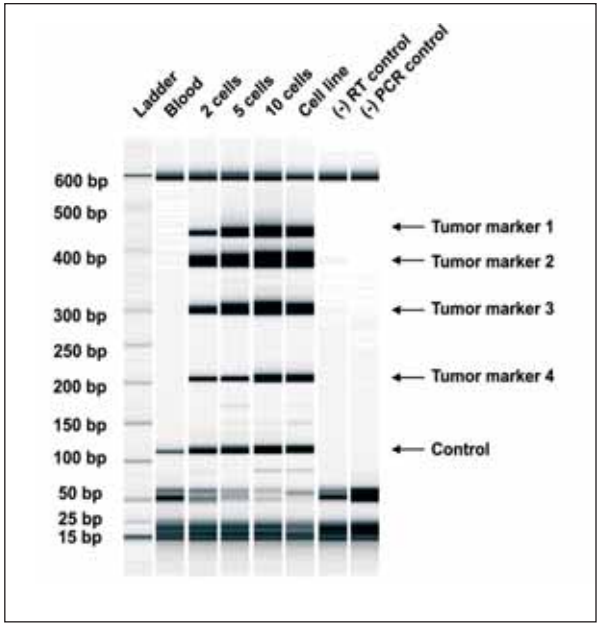
1 Low molecular weight marker
2 Primer-dimer in negative control
3 Soy lectin – 118 bp
4 35S GMO – 153 bp
5 Internal control – 217 bp
6 Corn zein – 278 bp
7 High molecular weight marker

Kit: DNA 1000 kit
Assay: DNA 1000 assay
Application: GMO detection by multiplex PCR is widely used for soy and corn. Often sequences from the transgene and species specific controls or internal standard are co-amplified by endpoint PCR in a screening procedure. Multiple products can be analyzed with the 2100 Bioanalyzer at high resolution and sensitivity. Quantification and comparison of product amounts may already lead to qualification of a positive screening result prior to analysis by expensive quantitative real time PCR.

Application note: 5989-0124EN

Oncology

Tumor cell detection from carcinoma patient blood



Data kindly provided by AdnaGen

Kit: DNA 500 kit*
Assay: DNA 500 assay*
Application: A combined method of specific tumor cell enrichment and a high sensitivity tumor cell detection by multiplex PCR allows analysis of several tumor marker genes. The method is so sensitive that it allows the detection of only a few tumor cells per 5 mL EDTA-blood. The 2100 Bioanalyzer provides the performance to detect the PCR products with high sensitivity and automated result flagging. This method offers new possibilities for monitoring and prognosis in routine diagnosis, and may facilitate an appropriate selection of patients for adjuvant therapy.

Application note: 5988-9341EN

* replaced with DNA 1000 kit and assay

Oncology

SNP analysis in cancer related P16 gene

Sample	1	2	3	4	5	6	7	8	9	10	11
Genotype316	C	C	CG	CG	CG	CG	CG	C	C	C	G
Genotype356	C	C	CT	CT	CT	C	C	CT	CT	T	C
gel-like view Agilent 2100 bioanalyzer											
Main Band	198	198	197	198	198	198	198	198	199	198	195
Extra Band 1			204	204	204						
Extra Band 2			216	215	215	214	214				

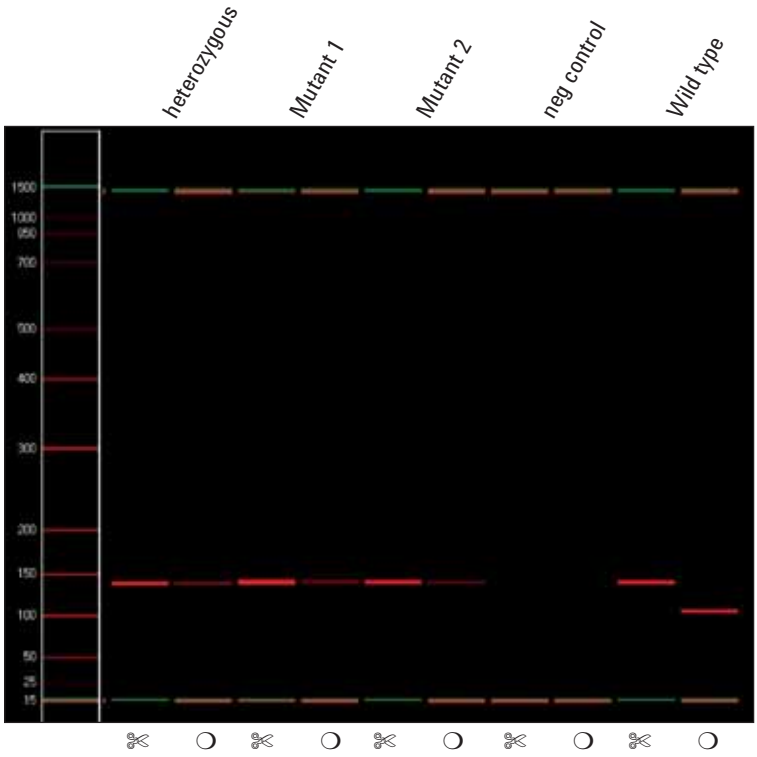
Data kindly provided by SAIC-Frederick

Kit: DNA 1000 kit
Assay: DNA 1000 assay
Application: Mutations in the exon 3 region of P16 gene are closely related to human cancer. A PCR yields 198 bp fragments with single, expected bands or additional, multiple bands in the 2100 Bioanalyzer analysis. These observations correspond perfectly to genotyping sequencing data of normal and mutant tissues. The pattern of bands is visible due to slower mobility of the heteroduplex formed by heterozygote mutant of the samples. The method provides fast and reliable acquisition of genetic diagnostic data from cancer patients, also on single nucleotide polymorphisms (SNP).

Application note: 5989-0487EN

Oncology

K-ras gene SNP detection



Data kindly provided by SAIC-Frederick

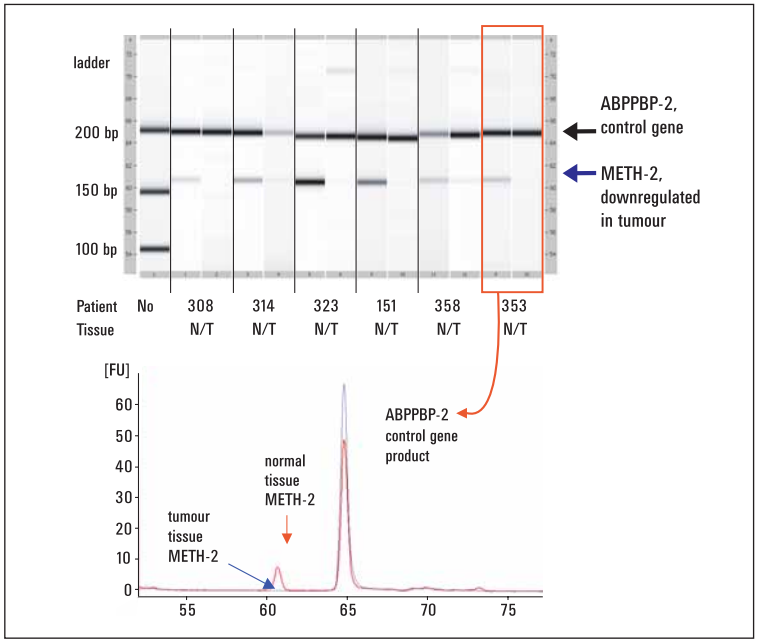
✂ – Restriction
❍ – PCR product

Kit: DNA 1000 kit
Assay: DNA 1000 assay
Application: Mutations in the K-ras gene coding 12 region can lead to cancer in different human tissues. A dedicated combination of PCR and specific restrictions (BstNI digest) reveals the underlying single nucletide polymorphisms (SNPs). The integral element within this test is the rapid and precise analysis of short amplicons (135 bp, see PCR-product lanes above) and fragments (106 bp, visible in lanes labeled with restriction) with the lab-on-a-chip technique. The test was used to ultimately determine a cancer patient's eligibility for a clinical trial for a peptide vaccine.

Application note: 5989-0487EN

Oncology

METH-2 downregulation in lung carcinomas



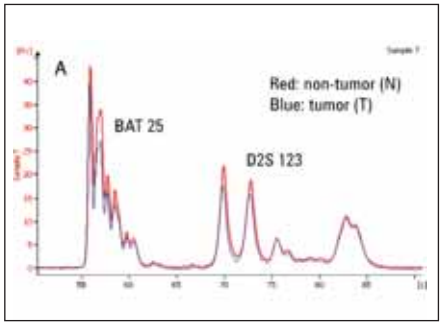
Data kindly provided by Roy Castle Lung Cancer Research Programme, University of Liverpool, UK

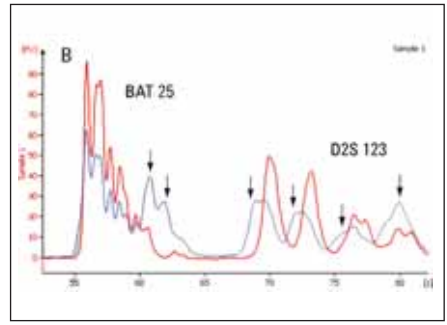
Kit: DNA 1000 kit
Assay: DNA 1000 assay
Application: Microarray analysis reveals under- or over-representations of transcripts. Screening of several cell lines for independent validation of such observations can be done with different techniques such as comparative multiplex PCR. This application shows the downregulation of a characteristic antiangiogenetic factor (METH-2) for a series of patient samples. Expression in normal tissue and tissue from the non small lung carcinomas is compared. Results from the array experiments were confirmed on a broad basis. Fast and convenient analysis with the 2100 Bioanalyzer with given quantitation capability fit perfectly in such analytical workflow.

Application note: 5989-3514EN

Oncology

Label-free analysis of microsatellite instability in carcinoma



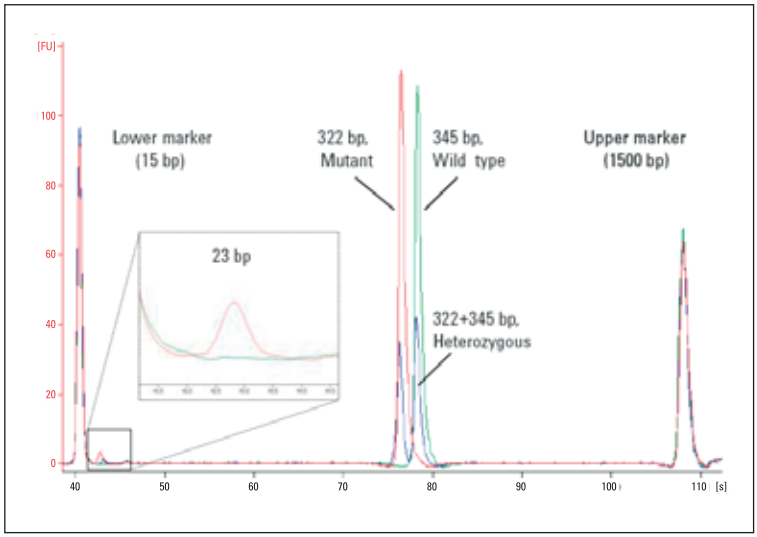


Kit: DNA 1000 kit
Assay: DNA 1000 assay
Application: Microsatellite instability (MSI) is caused by a failure of the DNA mismatch repair system and occurs frequently in various types of cancer. Given that conventional techniques used for MSI detection, for example, polyacrylamide gel electrophoresis (PAGE) or capillary electrophoresis, turned out to be laborious or expensive, this study aimed to develop a simple and efficient procedure of MSI detection. Detection of MSI could be demonstrated by microsatellite loci-associated, well defined deviations in the electropherogram profiles of tumor and non-tumor material and confirmed the classification of the MSI cases performed by conventional technology (95 % concordance rate). Whereas the results of the MSI detection were comparable to conventional techniques, the on-chip electrophoresis on the 2100 Bioanalyzer was superior in terms of speed, usability and data management.

Application note: 5989-2626EN

Clinical research

Detection of a point mutation in the prothrombin gene with PCR-RFLP

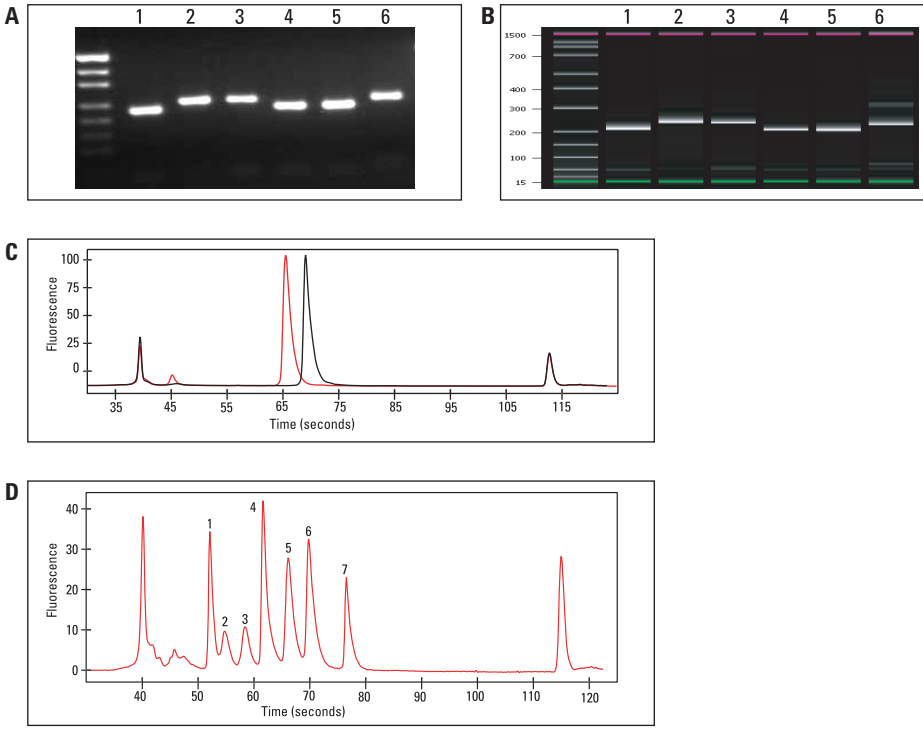


Kit: DNA 1000 kit
Assay: DNA 1000 assay
Application: A single nucleotide polymorphism (SNP), i.e. a point mutation in the prothrombin gene results in a common hereditary predisposition to venous thrombosis. PCR-RFLP was used for reliable detection of a specific SNP in the prothrombin gene. This SNP allows the introduction of a new Hind III restriction site during PCR. If the Hind III restriction site is present this is evidence for prothrombin G20210A mutation. Heterozygous (two bands) and homozygous (one band) genotypes can be specifically identified. The two fragments potentially generated in this PCR-RFLP protocol are only slightly different in size and are difficult to separate with traditional slab gel electrophoresis, even if optimized separation time of 2 hours is applied.
In contrast, the 2100 Bioanalyzer and the DNA 1000 kit allow precise baseline resolved separation of the expected fragments of 345 and 322 bp. The visualization of the 23 bp fragment cleaved off during the PCR-RFLP protocol is also possible, which further confirms a successful reaction. This is not possible with agarose gel electrophoresis. The results obtained with the 2100 Bioanalyzer are superior in terms of sizing accuracy, quantification capability, reproducibility and resolution compared to slab gel analysis.

Application note: 5989-4313EN

Clinical research

Genotyping of H. pylori



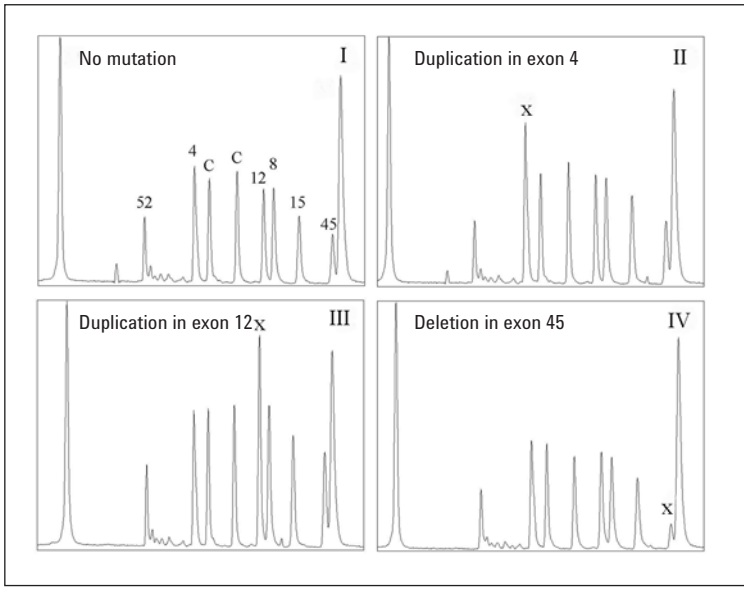
Data kindly provided by Institute for Pathology, Cologne

Kit: DNA 1000 kit
Assay: DNA 1000 assay
Application: Different allelic variants are associated with different stages of H. pylori virulence. Multiplex PCR on five alleles with products in the range of 102 to 301 bp were used to analyze DNA from paraffin embedded tissues. Agarose gel (A) yields only limited distinctiveness, whereas gel-like images (B) and electropherograms (C) show good resolution and superior reproducibility allowing convenient analysis of all desired products in parallel (D). An extended spectrum of prognostic or therapeutic relevant information is now routinely accessible for simultaneous analysis.

Application note: 5989-0078EN

Clinical research

Duplications and deletions in genomic DNA



Data kindly provided by Center for Human and Clinical Genetics, Leiden

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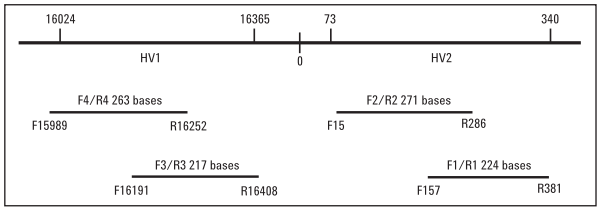
Kit: DNA 500 kit*
Assay: DNA 500 assay*
Application: Multiplex amplifiable probe hybridization (MAPH) and multiplex ligation-dependent amplification (MLPA) are high throughput techniques for the detection of reordered genomic segments. These methods include hybridization of amplifiable probes with either stringent washing or ligation events prior to amplification. Exact and reproducible sizing and quantitation of multiple products are important prerequisites which are delivered by the 2100 Bioanalyzer and lead to quick and simple analysis of genetically related diseases.

Application note: 5989-0192EN

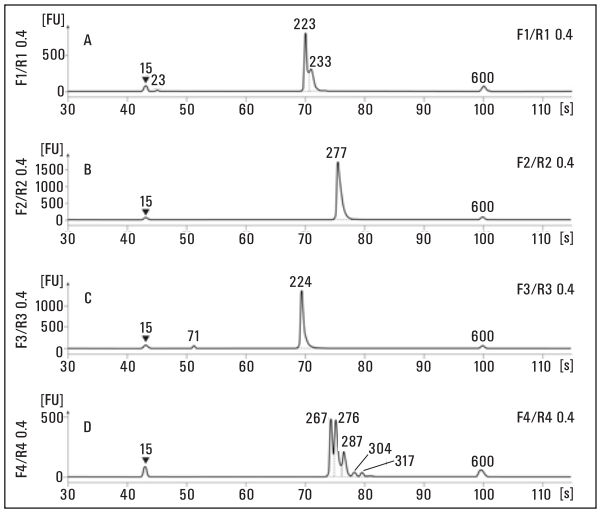
* replaced with DNA 1000 kit and assay

Forensic testing

Optimization of PCR on mtDNA



Amplified areas in human mtDNA



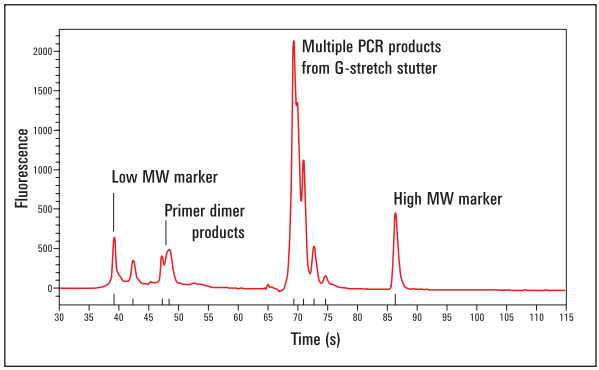
Kit: DNA 500 kit*
Assay: DNA 500 assay*
Application: Human mitochondrial DNA (mtDNA) is amplifiable even from small or badly degraded samples, even if genomic DNA is not available. Lanes B and C show homogenous PCR products which can subsequently be sequenced for identification. However, careful optimization of PCR parameters, like pH, Mg^{2+} concentration or polymerase amount is necessary and shown in detail in this application note. For example, a high Taq concentration increased the yield but also increased the level of byproducts. PCR for samples in lane D (impurities) and lane A (C-heteroplasmy) need to be improved. The 2100 Bioanalyzer provides a rapid quantitative analysis over the broad size and concentration range needed for optimization and QC. It has proven to be an indispensable tool for forensic labs.

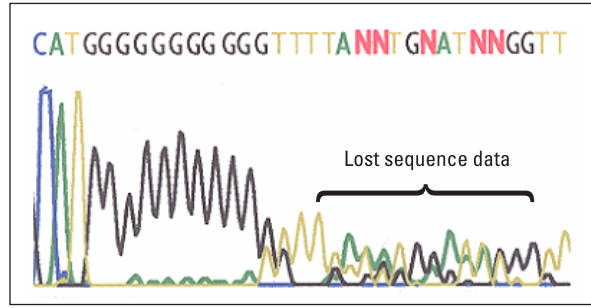
Application note: 5989-3107EN

* replaced with DNA 1000 kit and assay

Forensic testing

Pitfalls in mtDNA sequencing





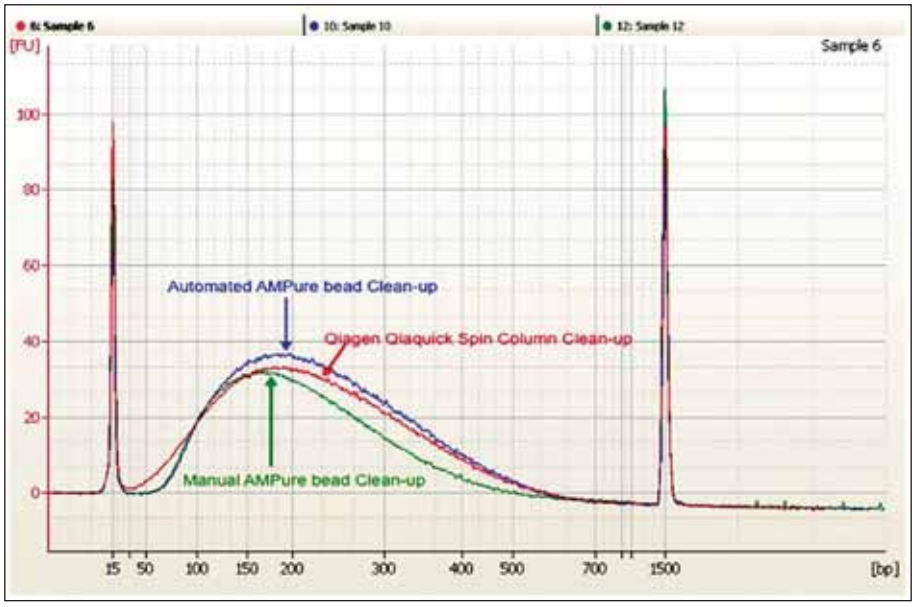
Kit: DNA 500 kit*
Assay: DNA 500 assay*
Application: Analysis of the non-coding sequence of human mitochondrial DNA (mtDNA) is performed for the purpose of identification in forensics. PCR amplification of limited or degraded mtDNA is done prior to sequencing. Quantitation and quality control of these PCR products (10-100 ng/mL, homogenous fragment in the range of 200-500 bp) was performed. Difficult PCR templates may cause G-stutters or other unintended byproducts of higher or lower mass (left). This may lead to indistinct sequence readings (right). Therefore, e.g. FBI guidelines enforce a 10 % impurity level at the most. Fulfillment of this prerequisite can be satisfactorily verified with the 2100 Bioanalyzer.

Application note: 5989-0985EN

* replaced with DNA 1000 kit and assay

Next-generation sequencing

DNA library quantity and quality

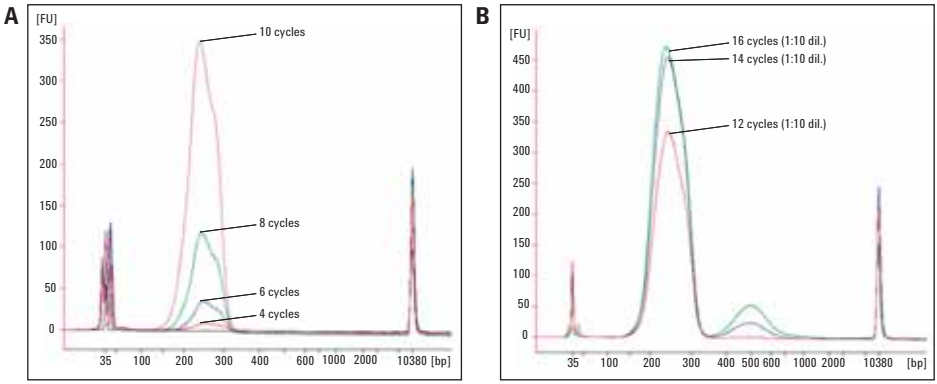


Kit: DNA 1000 kit
Assay: DNA 1000 assay
Application: An automated library construction protocol for the Illumina sequencing platform based on the Agilent Bravo Automated Liquid Handling Platform and the Agencourt Ampure PCR purification kit was used for next-generation sequencing and compared to other methods. The 2100 Bioanalyzer was used to quantify the amount of sample which was retained, as well as to analyze the purity of the sample. The overlaid 2100 Bioanalyzer traces are showing similar size distributions and sample purity using three different purification methodologies.

Application note: 5990-4942EN

Next-generation sequencing

DNA library QC in target enrichment and next-gen sequencing workflows



From [bp]	To [bp]	Corrected area	% of total	Average size [bp]	Size distribution in CV [%]	Concentration [pg/µL]	Molarity [pmol/L]
100	2,000	395.8	51	254	12.4	283.33	1,713.8

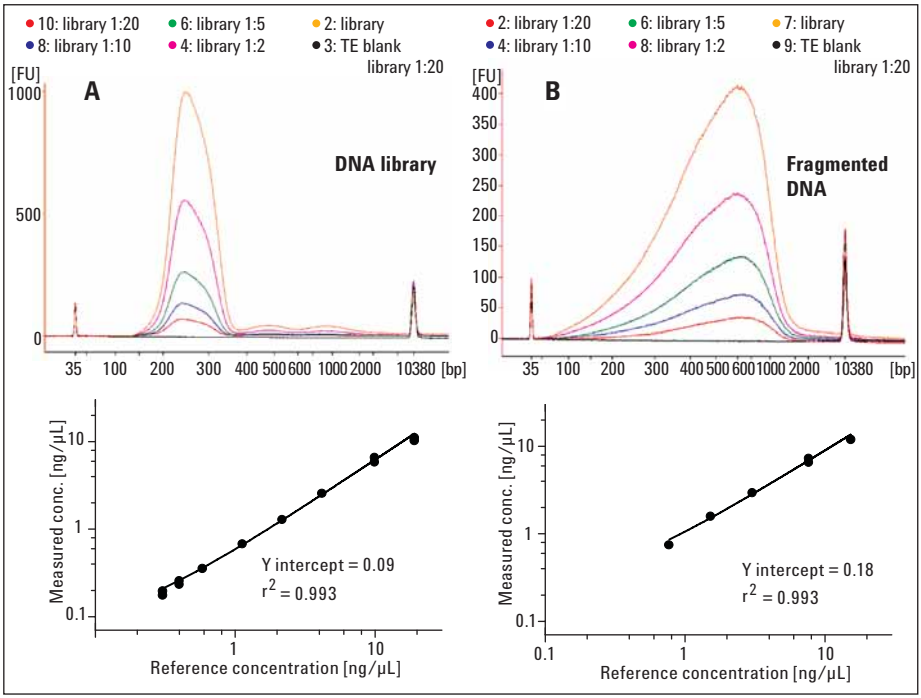
Quantification after 4 PCR cycles

Kit: High Sensitivity DNA kit
Assay: High Sensitivity DNA assay
Application: Here, the High Sensitivity DNA kit was used for quality control of amplified and purified DNA samples from the post-hybridization PCR amplification step prior to sequencing during the SureSelect Target Enrichment workflow. The electropherograms of typical PCR amplified DNA libraries show a typical smear from 150 to 350 nucleotides. The key observation clearly shown in figure 1B, is that the quality of the PCR product depended on the number of PCR cycles performed. After 14 PCR cycles, an additional DNA smear at approximately 500 bp was detected in the electropherogram. The excellent sensitivity of the High Sensitivity DNA kit allowed the amplified DNA to be detected and reliably quantified, even after only four PCR cycles. Thus, the numbers of library PCR cycles can be reduced, removing amplification bias and significantly improving the data quality with increased accuracy.

Application note: 5990-5008EN

Next-generation sequencing

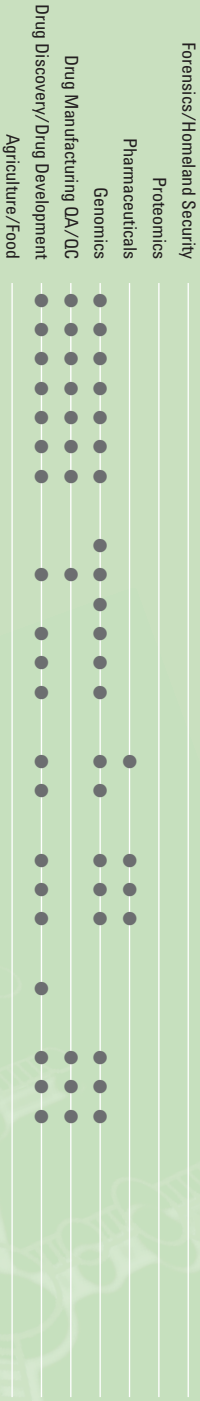
Sizing and quantitation of DNA libraries and fragmented DNA



Kit: High Sensitivity DNA kit
Assay: High Sensitivity DNA assay
Application: The High Sensitivity DNA kit provides sizing and quantification of DNA fragments and DNA smears in the 50 to 7000 bp size range down to pg/µL sensitivity. This is especially useful for sample quality control and the monitoring of critical steps in next-generation sequencing (NGS) workflows, including DNA fragmentation, target enrichment, and DNA library amplification. The analysis of a dilution series from two typical NGS samples, (A) Illumina DNA library and (B) fragmented DNA was performed. For both DNA sample types, the double logarithmic plot demonstrates an excellent linearity with $r^2 = 0.993$. This linear dynamic range depends on the library type and fragment distribution. The broad linear dynamic range of the High Sensitivity DNA kit enables the detection of less abundant products, such as PCR artifacts and impurities.

Application note: 5990-4417EN

III. RNA analysis



Analysis of total RNA
- Standardization of RNA Quality Control
- RNA integrity
- Standardization of RNA Quality Control
- Reproducibility of quantitation
- Genomic DNA contamination
- QC of automated RNA isolation from HeLa cells
- RNA quality from freshly frozen or formalin-fixed paraffin-embedded tissue

Low amounts of total RNA
- Detection of low levels of RNA
- RNA integrity with the RNA 6000 Pico kit
- RNA quality after staining and microdissection
- Analysis of minimum RNA amounts
- Genomic DNA in low concentrated RNA extracts
- Low RNA amounts from kidney sections

Analysis of mRNA
- RNA integrity
- Ribosomal RNA contamination in mRNA samples

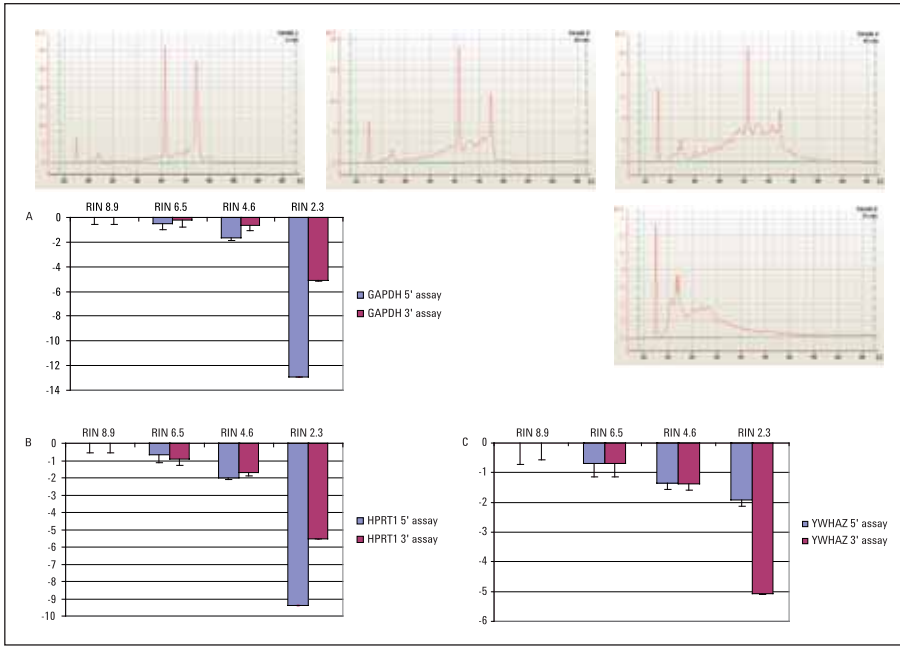
Analysis of Cy5-labeled samples
- Analysis of cRNA with and without dye in gel matrix
- Optimization of labeling reactions
- cRNA fragmentation

Analysis of T7-RNA transcripts
- Size estimation

Analysis of Small RNA
- Analysis of miRNA content in total RNA samples
- Analysis of small RNAs from Drosophila Schneider
- Effect of total RNA quality on mRNA and miRNA expression profiles

Analysis of total RNA

Standardization of RNA quality control

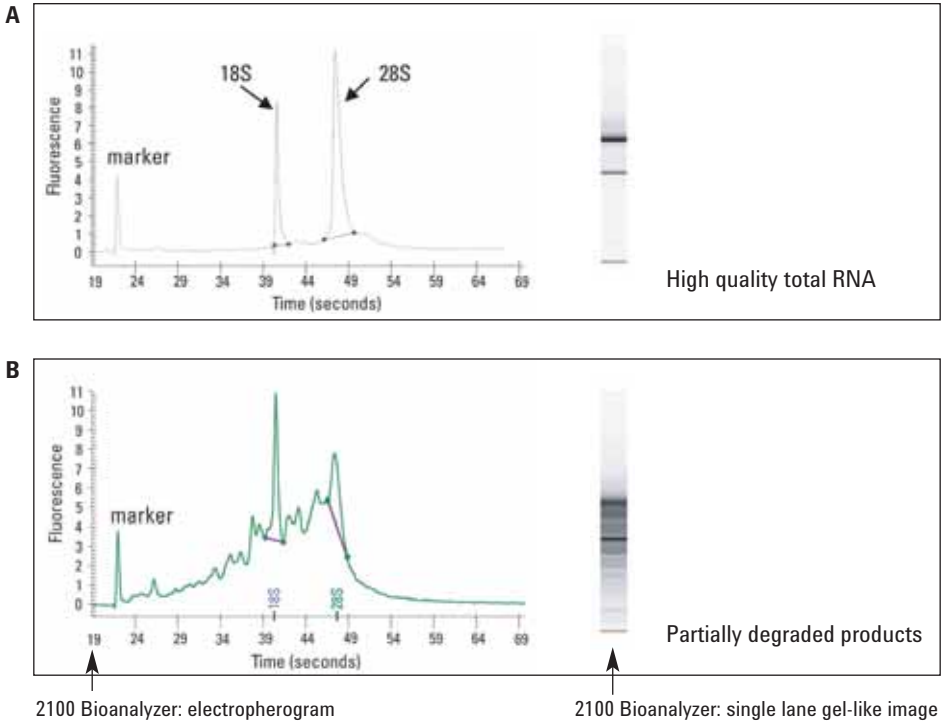


Kit: RNA 6000 Nano kit
Assay: Eukaryote Total RNA Nano assay
Application: Total RNA samples were degraded at 70 °C for varying times and were analyzed with the 2100 Bioanalyzer to determine the RNA integrity number (RIN). RIN is a reliable software tool to determine the integrity of RNA samples automatically.
The relative quantities of amplicons positioned at the 5' or 3' end of three target genes (GAPDH, HPRT1 and YWHAZ) differ when using differentially degraded RNA templates or highly intact RNA (RIN = 8.9). The data indicates that the integrity of RNA templates can significantly influence the outcome of a real-time quantitative PCR (QPCR) experiment. Furthermore, depending on the position of the amplicon major differences of the calculated fold-change in a comparative quantification can occur.

Application note: 5989-7730EN

Analysis of total RNA

RNA integrity

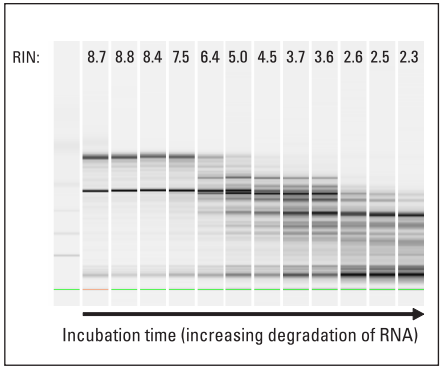


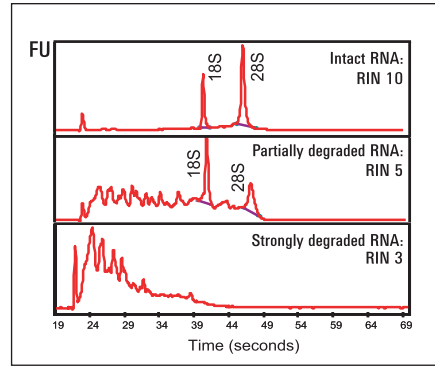
Kit: RNA 6000 Nano kit
Assay: Eukaryote Total RNA Nano assay
Application: Analysis of total RNA integrity – a typical first QC step during cDNA or cRNA sample prep for microarrays. In Figure A the upper electropherogram and gel-like image show the analysis of high quality total RNA with the 18S and 28S subunit as two distinct bands. Figure B shows the analysis of a partially degraded total RNA sample. Many degradation products appear between the two ribosomal bands and below the 18S band. With the help of the 2100 Bioanalyzer and the RNA 6000 Nano kit the important sample QC step prior to an expensive microarray experiment can be easily and quickly achieved.

Application note: 5968-7493EN

Analysis of total RNA

Standardization of RNA quality control





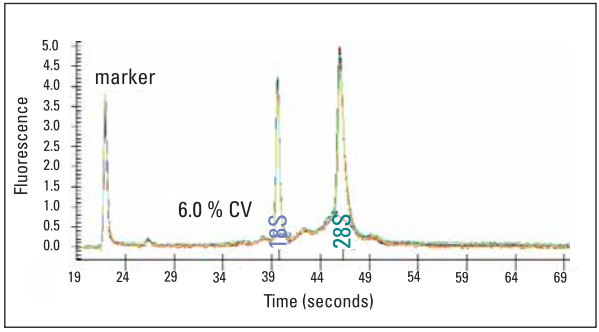
Kit: RNA 6000 Nano kit
Assay: Eukaryote Total RNA Nano assay
Application: The RNA integrity number (RIN) is calculated by a dedicated software algorithm to assess the quality of RNA preparations. The RIN tool is a major step in the standardization of user-independent RNA evaluation and delivers more meaningful information than simple ratio calculations for ribosomal RNA peaks. It is not influenced by instrument, sample integration and most important, concentration variability, thereby facilitating the comparison of samples and avoiding cost-intensive experiments with low quality RNA preparations. The RIN algorithm is based on a large collection of RNA data of various tissues and qualities. Furthermore, anomalies like genomic DNA contaminations are indicated with weighted error messages (critical/non-critical) to achieve a maximum of reliability.

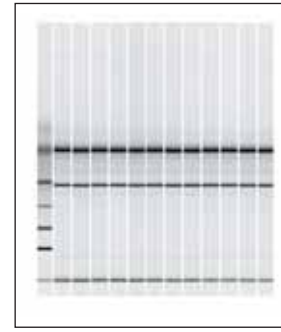
Application note: 5989-1165EN

Analysis of total RNA

Reproducibility of quantitation

Reproducibility for 12 consecutive runs





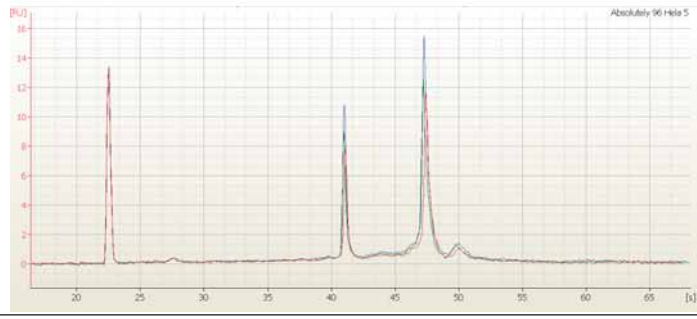
Kit: RNA 6000 Nano kit
Assay: Eukaryote Total RNA Nano assay
Application: Alongside the quality control of RNA samples, measurement of RNA concentration is important for (bio-)chemical reactions, such as labeling reactions in the context of microarray experiments. With the RNA 6000 Nano kit good reproducibility can be achieved (here 6 % CV), which is little affected by sample contaminants, such as phenol.

Application note: 5988-7650 EN

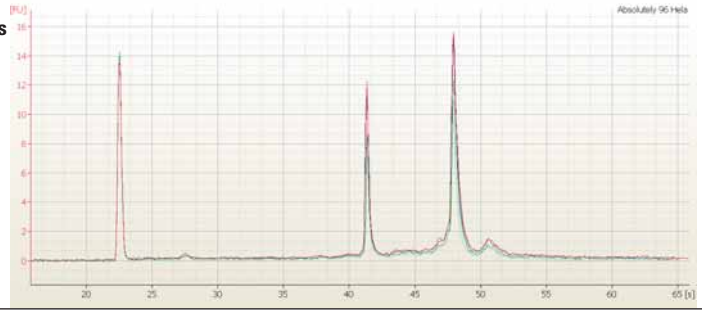
Analysis of total RNA

QC of automated RNA isolation from HeLa cells

RNA from middle row



RNA from 4 corners

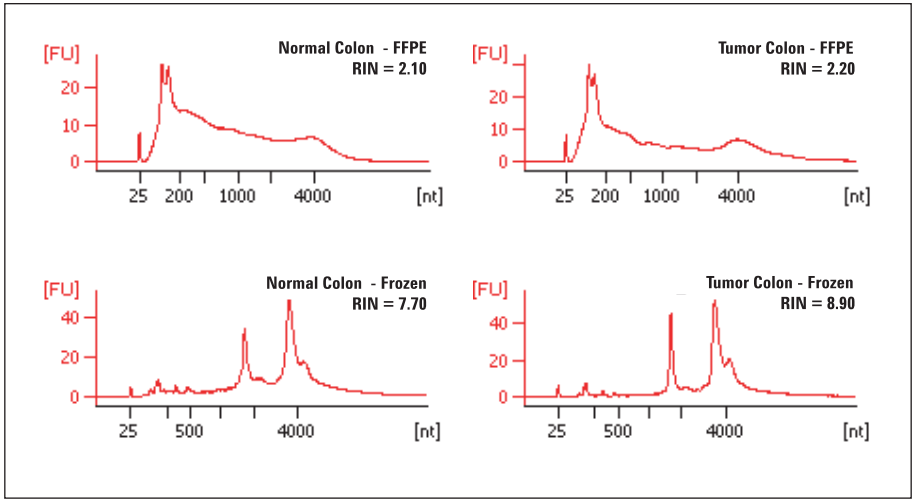


Kit: RNA 6000 Nano kit
Assay: Eukaryote Total RNA Nano assay
Application: An automated RNA isolation protocol in a 96-well format employing the Bravo Automated Liquid Handling platform and Absolutely RNA 96 Microprep kit from Agilent was used. The total RNA quality of 12 samples isolated from HeLa cell culture was assessed on the 2100 Bioanalyzer with the RNA 6000 Nano kit. Representative electropherograms of samples from the four corners (top) and the middle row (bottom) from the 96-well tissue culture dish are overlaid. The RNA is fully intact with sharp peaks for 18S and 28S ribosomal RNA. Furthermore, the presence of small RNA, and the absence of genomic DNA contamination can be observed. The RNA integrity numbers ranged from 9.2 to 9.6.

Application note: 5990-3558EN

Analysis of total RNA

RNA quality from freshly frozen or formalin-fixed paraffin-embedded tissue

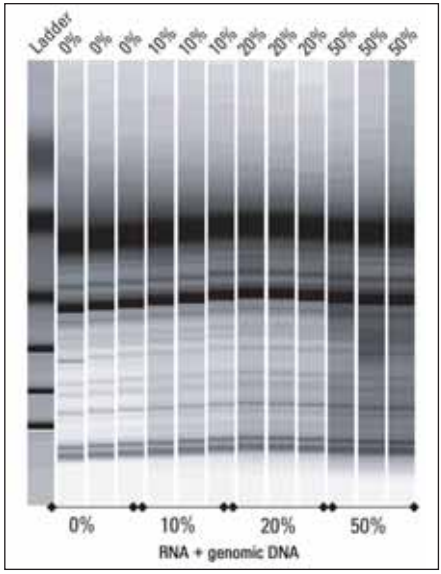


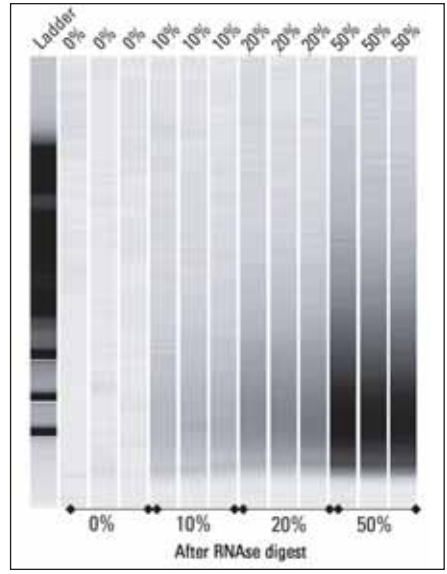
Kit: RNA 6000 Nano kit
Assay: Eukaryote Total RNA Nano assay
Application: RNA extracted from freshly frozen tissues is optimal for microarray analysis; however, in many cases, formalin-fixed paraffin-embedded (FFPE) tissues are the only samples available. Here, the effectiveness of an FFPE sample protocol optimized for use with Agilent gene expression microarrays was verified. For this purpose, total RNA was extracted from FFPE or fresh frozen tissues from colon tumor (adenocarcinoma) and normal colon and subjected to microarray analysis. The RNA quality was determined with the 2100 Bioanalyzer. The majority of RNA fragments isolated from the FFPE tissues were between 100–4,000 bp. The low RNA integrity number (RIN ~2.0), indicating degraded RNA, was typical for FFPE extractions as shown in the top two traces.
However, the quality of the gene expression information generated from FFPE samples and from high quality fresh frozen RNA was comparable. Despite the expected loss of sensitivity when analyzing degraded RNA, the differential expression results generated with FFPE and fresh frozen samples are highly concordant.

Application note: 5990-3917EN

Analysis of total RNA

Genomic DNA contamination



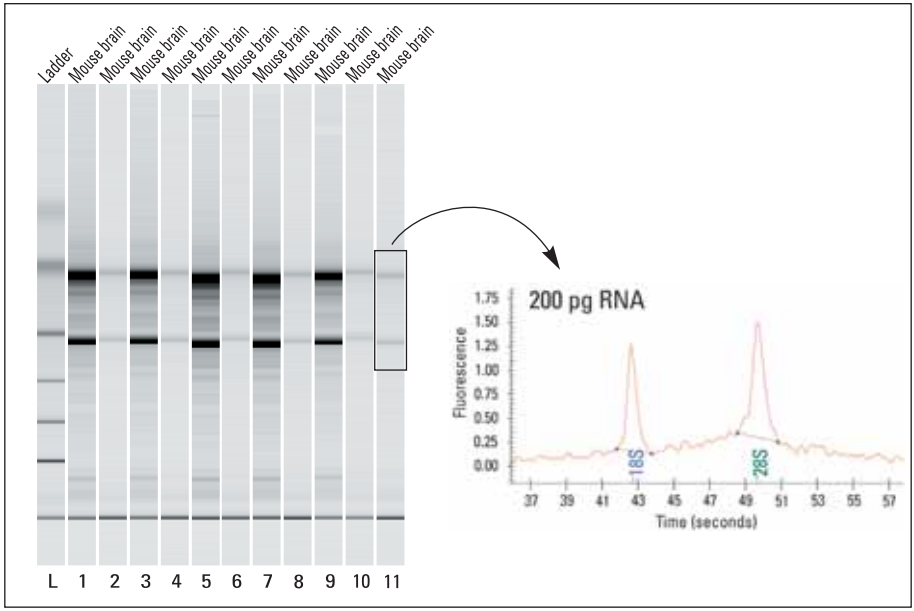


Kit: RNA 6000 Nano kit
Assay: Eukaryote Total RNA Nano assay
Application: Gel representation of a chip run with total RNA samples (mouse brain) spiked with varying amounts of herring sperm genomic DNA before and after treatment with RNase. The left panel shows the intact RNA with broad bands in the low MW region stemming from the genomic DNA. After the RNase digest (right panel) only the DNA bands remain, ranging in intensity according to the amount of DNA spiked into the sample.

Data not published

Low amounts of total RNA

Detection of low levels of RNA



Analysis of mouse brain RNA at two different concentrations

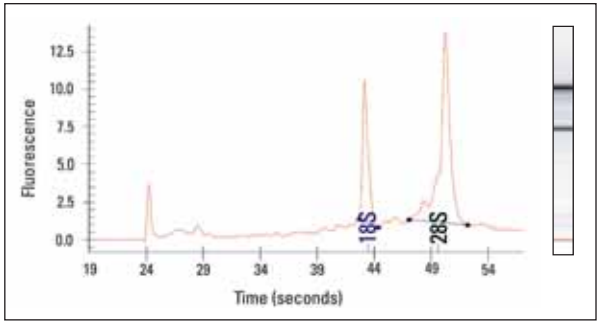
Kit: RNA 6000 Pico kit
Assay: Eukaryote Total RNA Pico assay
Application: The RNA 6000 Pico kit is complementary to the RNA 6000 Nano kit and is suitable for all applications where the amount of RNA (or cDNA) is limited, e.g. for biopsy samples, samples from microdissection experiments, QC of cDNA made from total RNA, microarray samples, etc. Here 2100 Bioanalyzer results obtained from mouse brain RNA (Ambion) at 200 and 1000 pg/µL are shown. By analysis in repetitions the reproducibility of quality control is demonstrated. Detection of 200 pg total RNA could be achieved without problems.

Data not published

Low amounts of total RNA

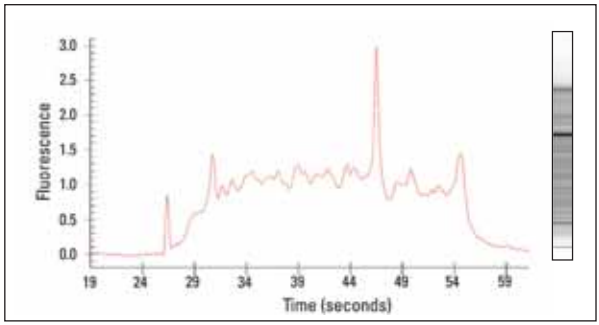
RNA integrity with the RNA 6000 Pico kit

A



Intact RNA

B



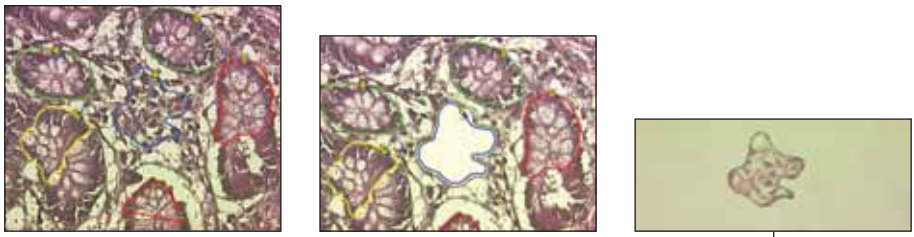
Degraded RNA

Kit: RNA 6000 Pico kit
Assay: Eukaryote Total RNA Pico assay
Application: Detection of RNA degradation with the RNA 6000 Pico kit. Sample: mouse liver total RNA (Ambion) concentration: 1 ng. Degradation was accomplished by adding a low amount of RNase. In Figure A the upper electropherogram and gel-like image show the analysis of high quality total RNA with the 18S and 28S subunit as two distinct bands. Figure A shows the analysis of a partially degraded total RNA sample. Many degradation products appear between the two ribosomal bands and below the 18S band.

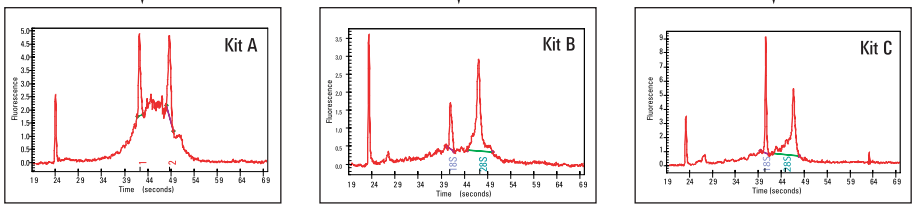
Data not published

Low amounts of total RNA

RNA quality after staining and microdissection



Laser micro dissection



Check and optimize RNA quality and yield

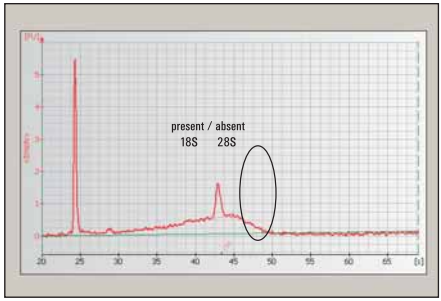
Kit: RNA 6000 Pico kit
Assay: Eukaryote Total RNA Pico assay
Application: RNA derived from laser-microdissected tissue isolated by the PALM®MicroBeam system was shown to be of high quality by convenient analysis with the RNA 6000 Pico assay. RNA-purification kits from different manufacturers and various common staining procedures have been tested and yielded 130-700 pg/µL RNA from 1000 cells with different quality (see above). The RNA 6000 Pico kit was well suited to show differences in RNA quality and yield and, therefore, is an ideal tool to optimize and adapt experimental conditions to individual tissue. The experiments were accompanied by a more laborious real time PCR that revealed similar results. Due to its unprecedented sensitivity, the RNA 6000 Pico assay is an indispensable tool for quality control in the context of microdissection experiments, ensuring successful gene expression profiling experiments.

Application note: 5988-9128EN

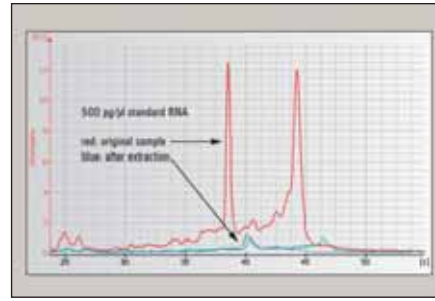
Low amounts of total RNA

Analysis of minimum RNA amounts

A



B



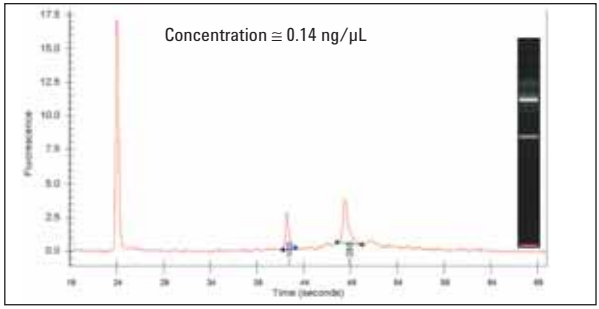
Kit: RNA 6000 Pico kit
Assay: Eukaryote Total RNA Pico assay
Application: The challenge of analysis of minimal amounts of RNA from e.g. laser micro dissections calls for detailed knowledge of extraction conditions. Some commonly used RNA isolation kits and buffer components were assessed in detail. The majority of the kits had no negative effect on the performance of the analysis, whereas, some kits include buffers which lead to shifted, missing and diminished RNA-peaks. In figure A, RNA isolated after microdissection shows lack of the 28S-peak due to high salt concentration introduced during the isolation process. In figure B, a standard RNA was diluted in water and subsequently extracted with a commercially available RNA extraction kit. The original samples (red) and the eluates after extraction are shown. These data show the importance of evaluating the individual method used for RNA extraction to exclude misleading conclusions.

Application note: 5989-0712EN

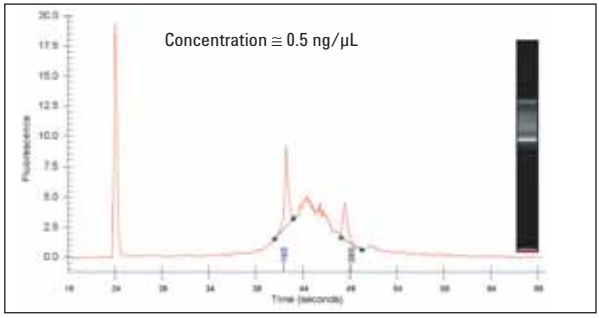
Low amounts of total RNA

Genomic DNA in low concentrated RNA extracts

A



B

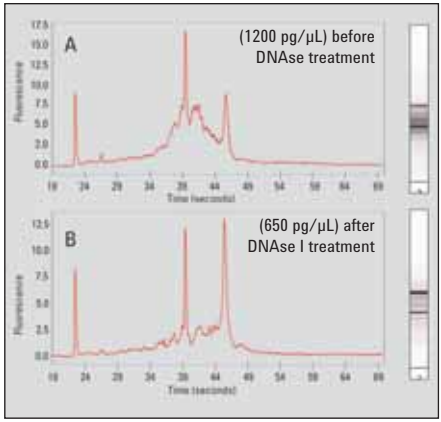


Kit: RNA 6000 Pico kit
Assay: Eukaryote Total RNA Pico assay
Application: Laser capture microdissection enables collection of cells from small tissue areas. A low RNA yield is in the nature of the extraction method from such a specimen that usually complicates quality assessment – a fact that can be circumvented by taking advantage of the 2100 Bioanalyzer capabilities. A comparative study using mouse kidney cryosections showed that on-column DNase digestion is indispensable to obtain a reasonable result for integrity and yield (figure A). Experiments with omitted on-column DNA digestion confirmed that the peak visible in the inter-region consists of genomic DNA which caused overestimation of extracted RNA amounts (figure B).

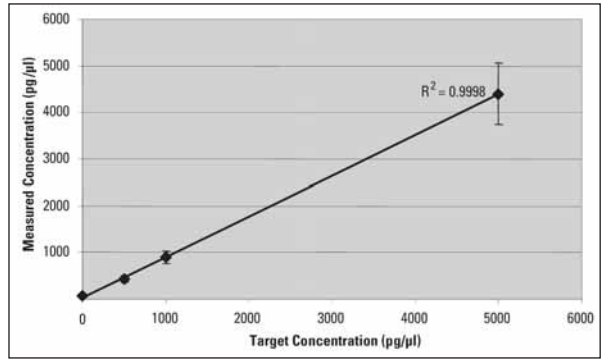
Application note: 5989-0991EN

Low amounts of total RNA

Low RNA amounts from kidney sections



Renal medulla RNA

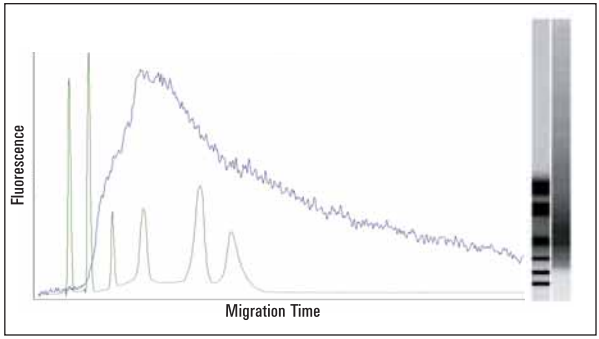


Kit: RNA 6000 Pico kit
Assay: Eukaryote Total RNA Pico assay
Application: High sensitivity quality control of RNA samples using the RNA 6000 Pico kit are demonstrated for microdissected samples (0.1 mm^3). DNAse I digestion revealed that DNA contamination was present in the sample. Removal of DNA revealed total RNA with a low degree of degradation. Under ideal conditions, the RNA Pico assay has a linear response curve and, therefore, allows estimation of RNA concentrations.

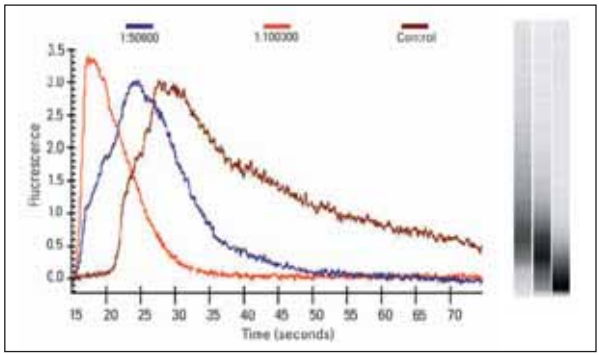
Application note: 5988-8554EN

Analysis of mRNA

RNA integrity



Highly enriched
Poly (A)+ RNA



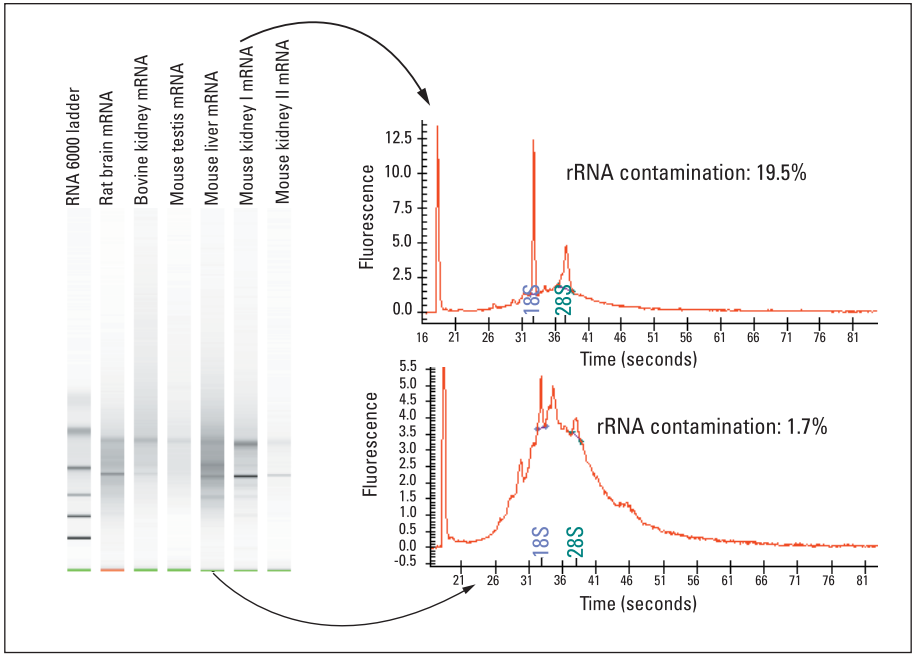
Progressive degradation
of Poly (A)+ RNA

Kit: RNA 6000 Nano kit
Assay: mRNA Nano assay
Application: Progressive degradation of Poly (A)+ RNA. Poly (A)+ RNA (60 ng/µL) from cultured Jurkat cells was incubated for 15 minutes at room temperature with very dilute RNase A (1 x 10^{-6} and 2 x 10^{-6} mg/mL, respectively). A progressive shift towards shorter fragment sizes can be observed. Even with a mild degradation, the absence of very long transcripts can be noticed.

Application note: 5968-7495EN

Analysis of mRNA

Ribosomal RNA contamination in mRNA samples

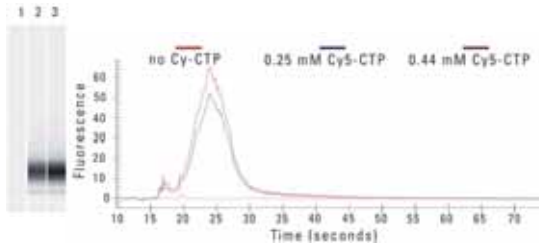


Kit: RNA 6000 Nano kit
Assay: mRNA Nano assay
Application: During the isolation of mRNA, varying amounts of ribosomal RNA can remain in a sample. Since the purity of mRNA is of importance for a number of downstream applications, samples should be checked on the 2100 Bioanalyzer. This slide shows the analysis of 6 commercially available RNA samples from different suppliers. Analysis on the 2100 Bioanalyzer reveals large differences in the purity of the mRNA samples.

Application note: 5968-7495EN

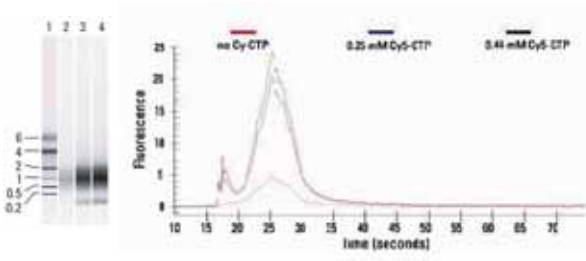
Analysis of Cy5 labeled samples

Analysis of cRNA with and without dye in gel matrix



Assay conditions:
- Cy5 labeled nucleic acids
- no intercalating dye used
- 5nM Cy5 dCTP added to gel matrix and sample buffer for focusing

Lanes:
1. Unlabeled cRNA
2. Cy5 labeled cRNA
3. Cy5 labeled cRNA



Assay conditions:
as above, but intercalating dye included in gel matrix

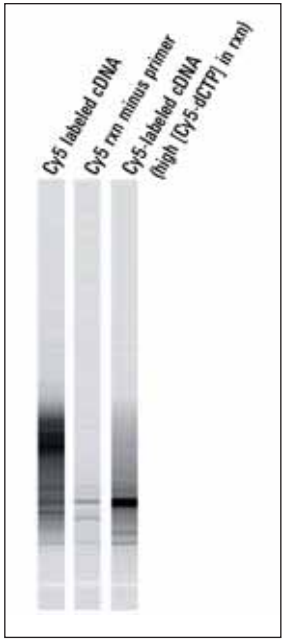
Lanes:
1. RNA transcript ladder
2. Unlabeled cRNA
3. Cy5 labeled cRNA
4. Cy5 labeled cRNA

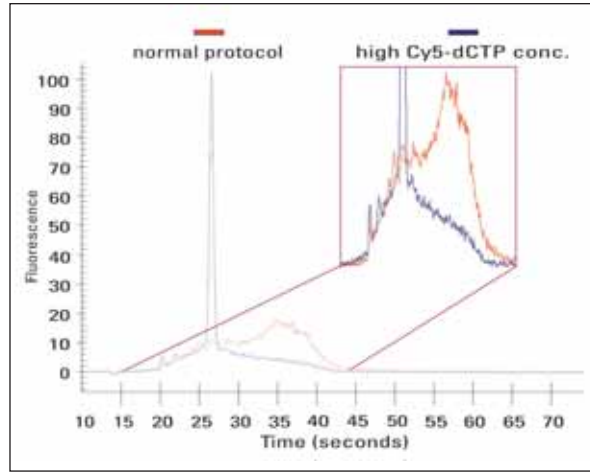
Kit: RNA 6000 Nano kit
Assay: mRNA Nano and Cy5 Labeled Nucleic Acids Nano assay
Application: Analysis of Cy5 labeled and non-labeled cRNA samples. Cy5-labeled samples show the combined signals of the fluorescent label and the RNA signal created by the fluorescence of the RNA 6000 dye. If the RNA 6000 dye is omitted from the gel matrix, only the signal created by Cy5 is detected, allowing the determination of dye incorporation after a labeling reaction. Please note that for Cy3 labeled samples the intactness of the sample can be verified, but the dye incorporation can not be checked.

Application note: 5980-0321EN

Analysis of Cy5 labeled samples

Optimization of labeling reactions



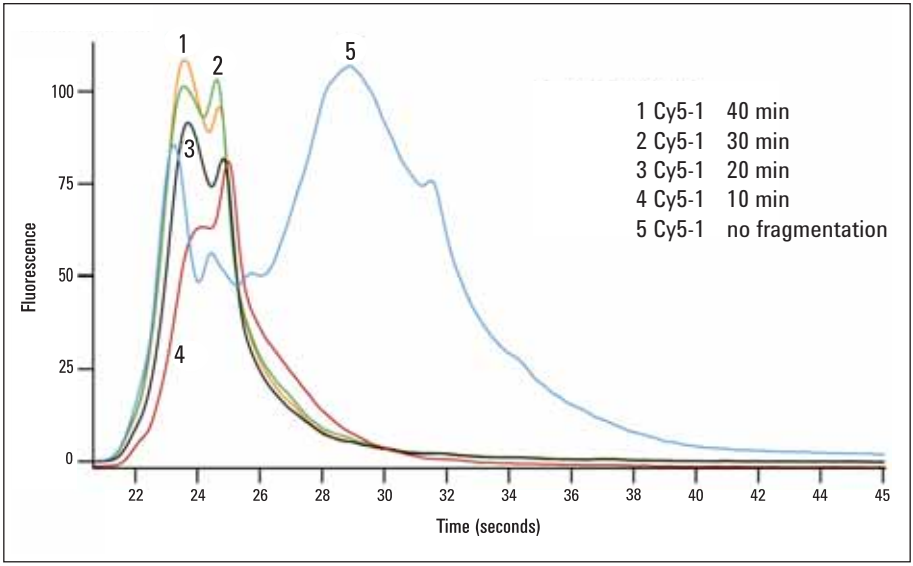


Kit: RNA 6000 Nano kit
Assay: Cy5 Labeled Nucleic Acids Nano assay
Application: An experiment was designed to check the influence of Cy5 dCTP concentration on labeling efficiency. Lane 2 represents the negative control (primer ommitted from the reaction mixture), while lane 3 shows the analysis of a reaction with a 6-fold increased Cy5 dCTP concentration. A look at the electropherograms reveals that the high Cy5 dCTP concentration not only gave a high peak of unincorporated Cy5, but also the labeling efficiency for longer fragments was very low. This approach allows the optimization of labeling reactions.

Application note: 5980-0321EN

Analysis of Cy5 labeled samples

cRNA fragmentation

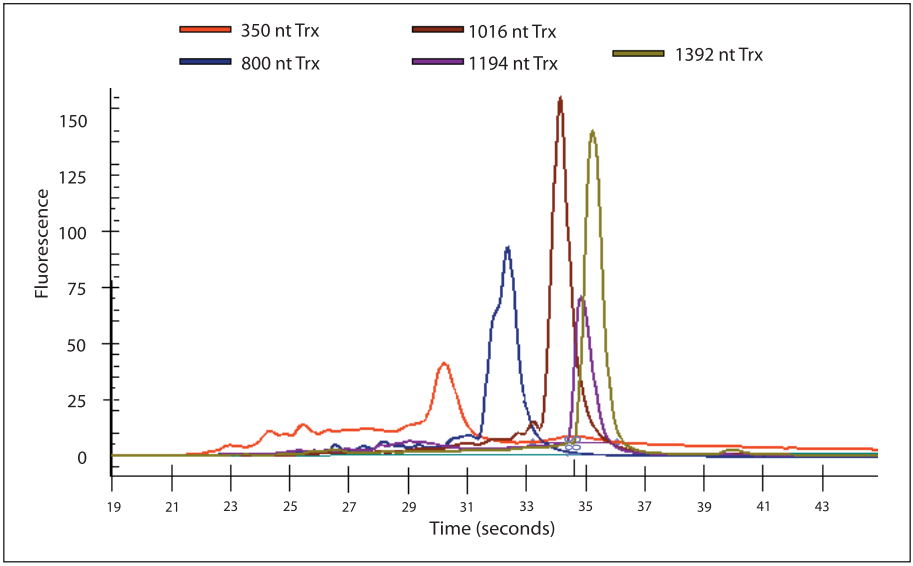


Kit: RNA 6000 Nano kit
Assay: Eukaryote Total RNA Nano assay
Application: The RNA 6000 Nano kit can be used to monitor completion of a cRNA fragmentation reaction. In this example, the profile of a Cy5 labeled cRNA sample was monitored at different time points during a fragmentation reaction. It can be seen that after 10 minutes most of the fragments are in the desired size range. After 20 minutes, no further shift of fragmentation can be observed indicating completion of the fragmentation reaction.

Application note: 5988-3119EN

Analysis of T7 RNA transcripts

Size estimation

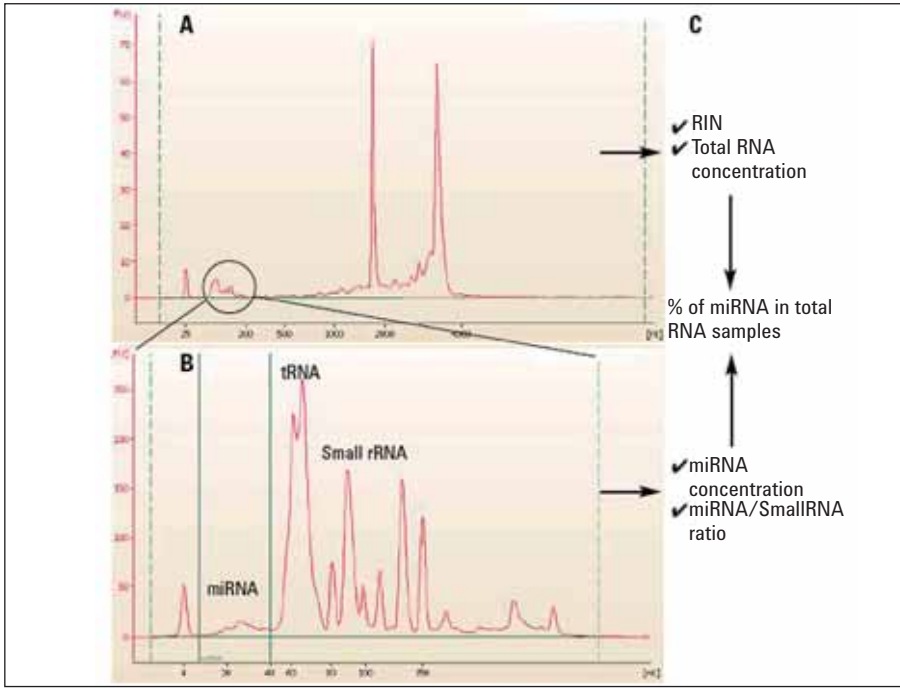


Kit: RNA 6000 Nano kit
Assay: Eukaryote Total RNA Nano assay
Application: A number of RNA transcripts, ranging from 350 to 1400 nt in size, were analyzed on the RNA 6000 Nano kit. Although the assay runs under native conditions and the transcripts exhibit a certain degree of secondary structure, a good size estimation can be achieved.

Data not published

Analysis of small RNAs

Analysis of miRNA content in total RNA samples



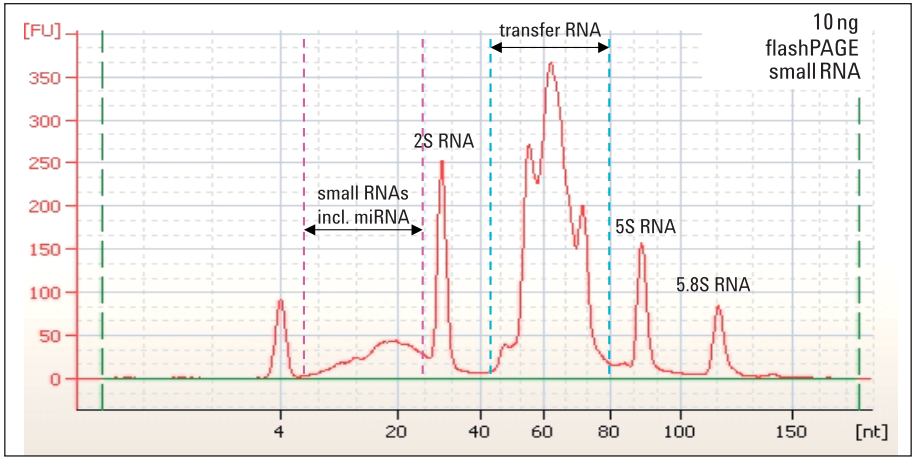
Kit: Small RNA and RNA 6000 Nano kit
Assay: Small RNA and Eukaryote Total RNA Nano assay
Application: Several total RNA samples containing small RNAs (including miRNAs, siRNA, and snRNA) were analyzed using the RNA 6000 Nano assay to determine concentration and quality, including RIN. All total RNA samples were then analyzed with the Small RNA assay to measure miRNA concentration. The relative amount of miRNA was manually calculated as a ratio of the concentration of miRNA in total RNA.

A) total RNA analyzed with the RNA 6000 Nano assay
B) small RNA analyzed with the Small RNA assay
C) miRNA analysis workflow

Application note: 5989-7870EN

Analysis of small RNAs

Analysis of small RNAs from *Drosophila* Schneider

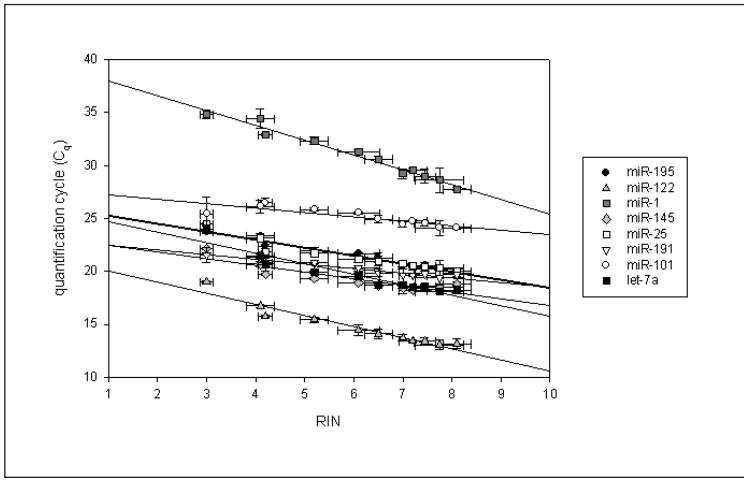


Kit: Small RNA kit
Assay: Small RNA assay
Application: Small RNA samples were prepared from Drosophila Schneider cells according to different protocols and were analyzed using polyacrylamide gel electrophoresis, northern blot, or the 2100 Bioanalyzer. Only 10 ng of total RNA was run on the Small RNA kit. Two sharp peaks, corresponding to 5.8S and 5S RNA, were clearly resolved. Transfer RNA (tRNA) formed a third large peak. A small well-defined peak at 30 nucleotides indicated that 2S RNA could be detected with only 10 ng of total RNA. Therefore, the Small RNA kit was significantly more sensitive than a denaturing 15 % polyacrylamide gel stained with ethidium bromide or SYBR Green II, respectively. Hence, the Small RNA kit appeared to be the method of choice to assess small RNA species contained in a total RNA preparation.

Application note: 5989-8539EN

Analysis of small RNAs

Effect of total RNA quality on mRNA and miRNA expession profiles



Kit: Small RNA kit
Assay: Small RNA assay
Application: Total RNA quality has a significant impact on quantitative PCR results. The MIQE guidelines also emphasize the importance of RNA quality assessment. The importance of RNA quality should also be considered for the investigation of miRNA expression profiles. Eight different miRNAs were quantified using reverse transcription quantitative PCR (RT-qPCR). For all tissues and in all eight genes, a highly significant, negative correlation ($p<0.001$) between the RNA integrity number (RIN) and the quantification cycle (Cq) occurred (see figure). With ongoing RNA degradation the Cq increased, showing an impairing influence of the RNA integrity on the performance of the qPCR. Analog experiments with mRNA showed that the expression analysis of miRNAs is influenced by RNA integrity to a lesser degree than mRNA expression. The RIN=5 threshold level for reliable PCR results for mRNA and miRNA was determined.

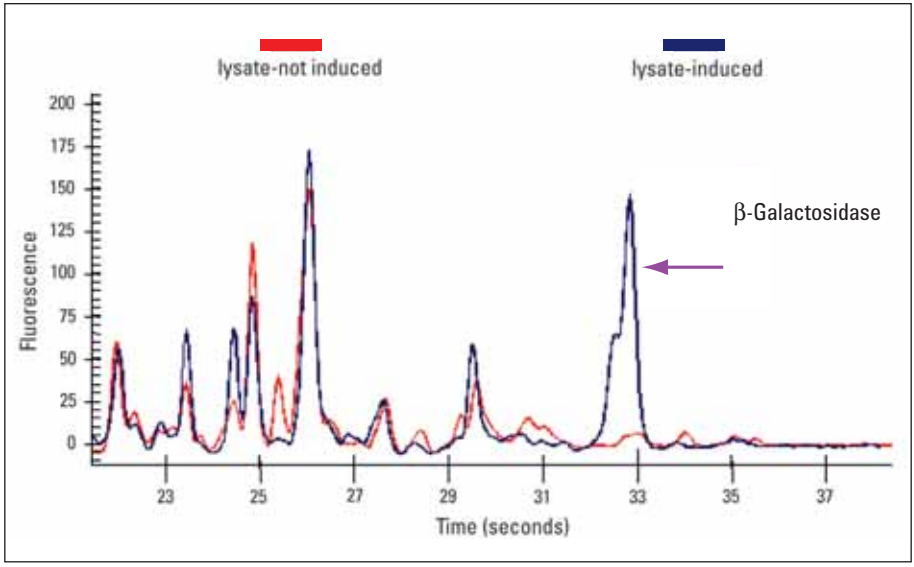
Application note: 5990-5557EN

IV. Protein analysis

	Agriculture/Food	Drug Discovery/Drug Development	Drug Manufacturing QA/QC	Genomics	Pharmaceuticals	Proteomics	Forensics/Homeland Security
Protein expression							
Analysis of cell lysates – protein induction			●			●	
Protein purification							
Comparison between lysate and flow through		●			●		
Analysis of protein purification		●		●		●	
GFP Streptag fusion protein purification		●				●	
Analysis of column capacity						●	
Analysis of column fractions to optimize conditions		●	●			●	
His-tag protein purification with Ni++ ZipTips®		●	●			●	
Determination of protein expression level and recovery			●		●		
Optimization of on-column cleavage			●		●		
Enzymatic removal of His-tags from recombinant proteins			●			●	
Complementing RP-HPLC protein purification		●			●	●	
High sensitivity protein detection							
Detection of low protein amounts		●	●				
Highly specific and sensitive alternative to Western blotting		●	●			●	
Pico labeling protocol for samples below 1 ng/µL protein		●	●			●	
Antibody analysis							
Analysis of antibodies under reducing and non-reducing conditions		●	●		●	●	
High sensitivity analysis of antibodies		●	●		●	●	
QA/QC of IgG under reducing conditions		●	●		●		
Quantitation of the half-antibody content in IgG_4 preparations		●	●		●		
Comparison of SDS-PAGE, CGE and 2100 Bioanalyzer for humanized monoclonal antibody analysis		●	●			●	
Absolute quantitation of IgG		●	●			●	
Quality control of stressed antibodies		●	●				
Separation of bispecific antibodies chains		●	●		●	●	
Analysis of IgG2 under non-reducing conditions		●	●		●		
IgG2 analysis under reducing conditions		●	●		●		
Monitoring antibody charge variants		●	●		●		
Separation of bispecific antibody chains		●	●		●		
Protein quantification							
Quantification of low protein amounts with an internal standard	●	●	●		●	●	
Absolute protein quantitation	●		●		●	●	
Food analysis							
Rapid wheat varietal identification	●					●	
Bovine milk analysis	●					●	
Protein pattern of different transgenic seedlines	●					●	
Protein – others							
Glycoprotein sizing	●		●			●	
OFFGEL electrophoresis combined with high-sensitivity on-chip protein detection	●	●	●		●	●	
Protein quality control prior to MS-analysis			●			●	
Depletion of high abundant proteins from blood samples		●			●		
Increased sensitivity by desalting protein samples	●	●			●	●	

Protein expression

Analysis of cell lysates – protein induction



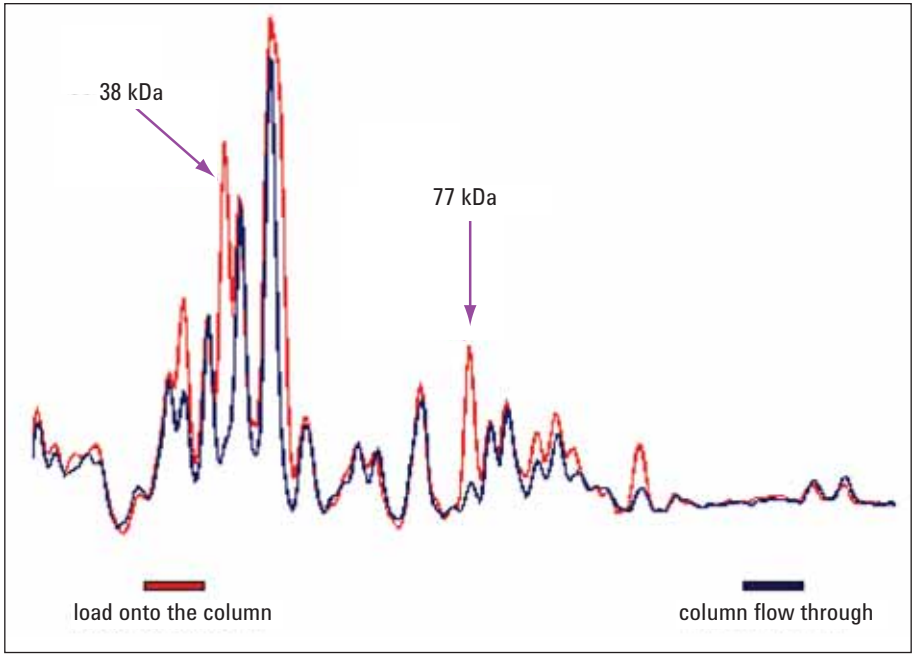
Kit: Protein 200 Plus kit*
Assay: Protein 200 Plus assay*
Application: Two cell lysates, induced and non-induced were compared to verify the induction of protein expression. The overlay feature of the bioanalyzer software allows quick sample comparison. The blue electropherogram trace shows the cell lysate highly expressing β-galactosidase (128 kDa).

Data not published

* replaced with Protein 230 kit and assay

Protein purification

Comparison between lysate and flow through



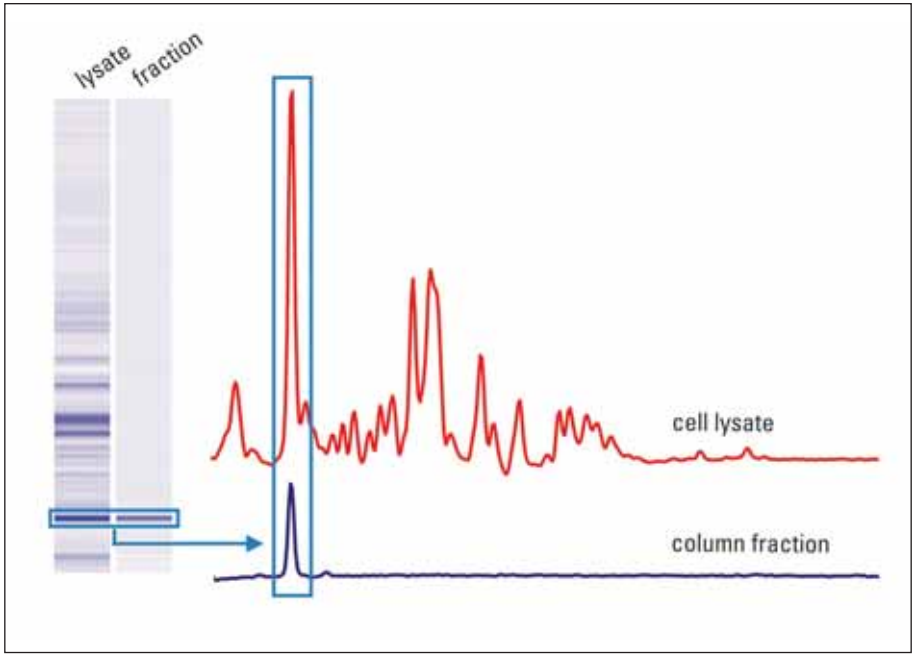
Kit: Protein 200 Plus kit*
Assay: Protein 200 Plus assay*
Application: Cells were lysed using the Pierce B-Per kit and then loaded onto an affinity column. The protein of interest, a 38 kDa protein, should bind to the column and not show up in the flow through. By overlaying the 2 electropherograms from both samples, the lysate and the flow through, it is visible that the protein of interest has bound to the column as expected. In addition, a 77 kDa protein has bound to the column, which could be attributed to unspecific binding or the binding of a dimer.

Data not published

* replaced with Protein 230 kit and assay

Protein purification

Analysis of protein purification



Courtesy of P. Sebastian and S.R. Schmidt GPC-Biotech AG, Martinsried, Germany

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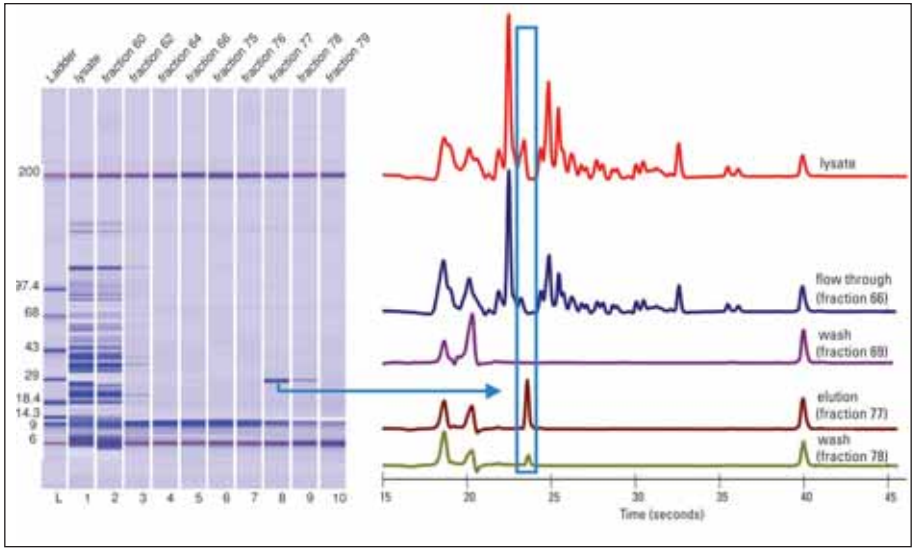
Kit: Protein 200 Plus kit*
Assay: Protein 200 Plus assay*
Application: A 18 kDa protein was purified using affinitiy chromatography. The starting material and the column fraction were analyzed with the protein assay. The protein of interest was determined to be 99 % pure and the concentration in the column fraction was 167 ng/µL. The protein assay allows protein purity and concentration to be determined in one step, in addition it calculates protein size for reconfirmation.

Data not published

* replaced with Protein 230 kit and assay

Protein purification

GFP Streptag fusion protein purification



Courtesy of P. Sebastian and S.R. Schmidt GPC-Biotech AG, Martinsried, Germany

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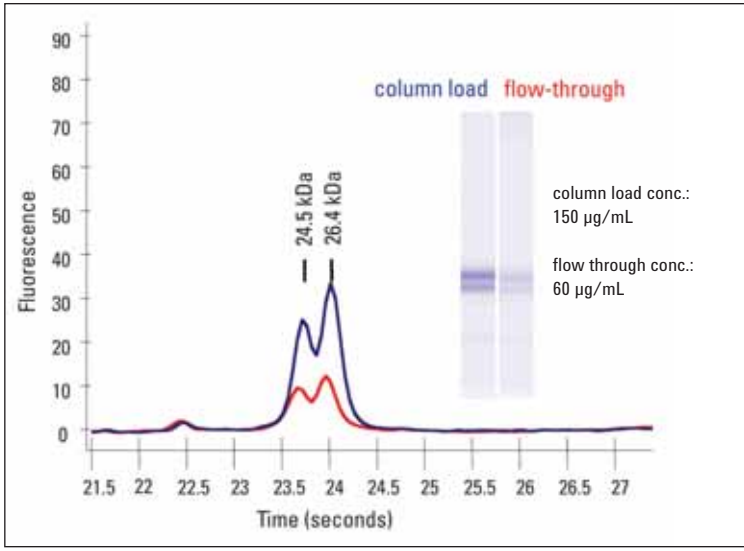
Kit: Protein 200 Plus kit*
Assay: Protein 200 Plus assay*
Application: This example shows the analysis of various steps during the purification workflow of a GFP Streptag fusion protein (28 kDa). The protein was expressed in *E.coli* and purified via affinity chromatography with Strep Tactin Poros as the column matrix. The protein assay allows each purification step from the cell lysis to the elution of the purified protein to be monitored and optimized.

Application note: 5988-5025EN

* replaced with Protein 230 kit and assay

Protein purification

Analysis of column capacity



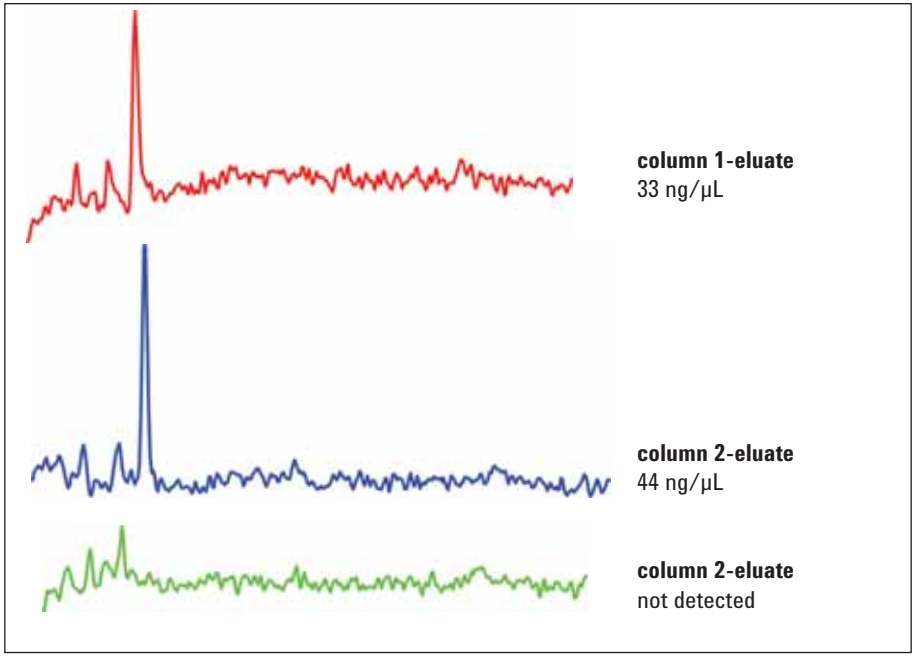
Kit: Protein 200 Plus kit*
Assay: Protein 200 Plus assay*
Application: The binding of a recombinant antibody Fab fragment to a Sepharose column with immobilized Protein G was analyzed to determine the column capacity and prevent column overloading. The protein assay allows this purification step to be monitored and quickly optimized.

Application note: 5988-4022EN

* replaced with Protein 230 kit and assay

Protein purification

Analysis of column fractions to optimize conditions



Courtesy of P. Sebastian and S.R. Schmidt GPC-Biotech AG, Martinsried, Germany

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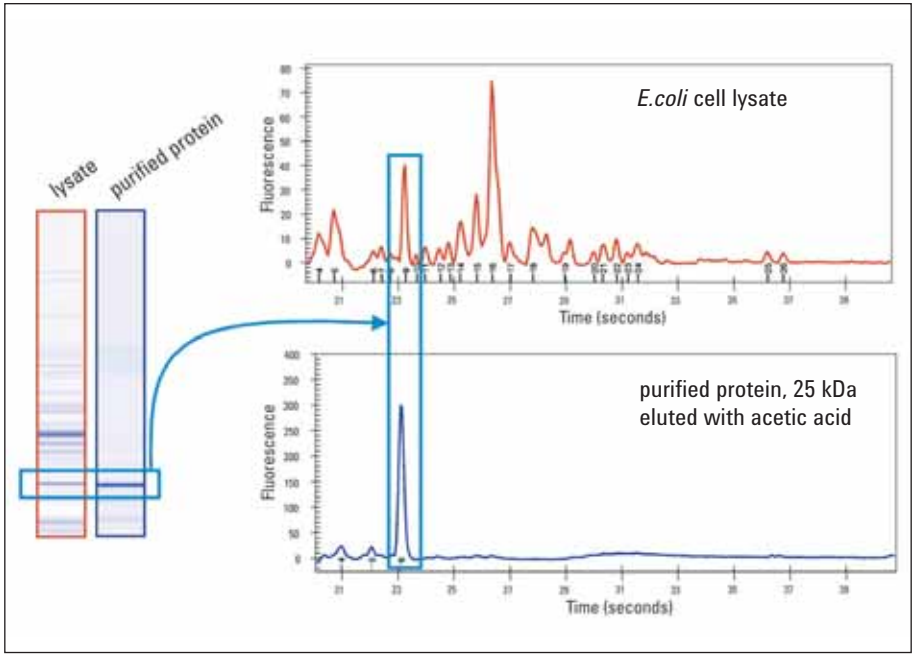
Kit: Protein 200 Plus kit*
Assay: Protein 200 Plus assay*
Application: Different column conditions were tested to optimize the purification conditions for a 30 kDa protein. The column fractions were analyzed for protein purity and concentration to identify the optimal conditions providing a highly purified protein in a good yield. Using the protein assay it was possible to determine the optimum purification conditions in a short time frame.

Data not published

* replaced with Protein 230 kit and assay

Protein purification

His-tag protein purification using Ni++ZipTips®



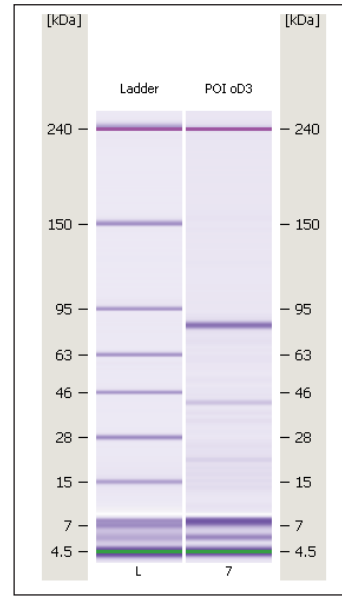
Kit: Protein 200 Plus kit*
Assay: Protein 200 Plus assay*
Application: ZipTips loaded with a Ni^{2+}-resin (in development by Millipore) were used to purify a His-tagged protein expressed in *E.coli.* Both the cell lysate and the purified protein were analyzed with the 2100 Bioanalyzer to demonstrate the performance of the tips. The purification with the tips takes approximately 5 minutes, usually followed by the analysis of the samples with SDS-PAGE analysis which takes a further 2 hours. The SDS-PAGE analysis was substituted by the much faster Protein 200 Plus* assay run on the 2100 Bioanalyzer.

Data not published

* replaced with Protein 230 kit and assay

Protein Purification

Determination of protein expression level and recovery



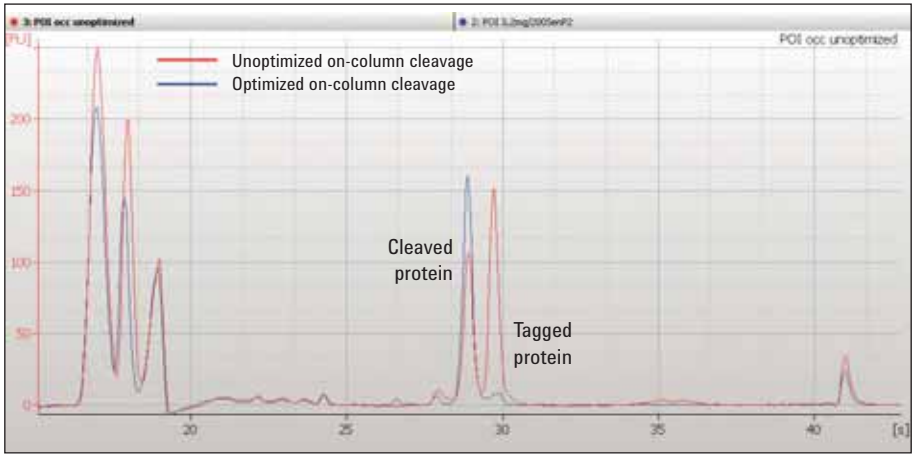
Sample	Rel. Conc. [ng/µL]	% Total	Volume [mL]	POI total [mg]
Total lysate	1,368.7	48.2	20	274
Lysate supernatant	551.2	38.2	20	110
Flow through	325.1	29.9	20	65
Wash 1	435.7	48.3	30	13
Wash 2	405.9	84.1	30	12
SenP2 cleavage	4,784.3	67.4	2	10
Imidazole eluate	9,745.4	55.2	1	10

Kit: Protein 230 kit
Assay: Protein 230 assay
Application: Monitoring the protein of interest (POI) during purification in a fast and reliable manner is fundamental. Here, protein purification of a His6-Sumo3-tagged DNA-binding protein was performed with Immobilized Metal Affinity Chromatography (IMAC). Protein expression and recovery was monitored with the Protein 230 assay. Based on the relative quantification with the 2100 Bioanalyzer, the overall yield for the POI was estimated to 30 mg/g biomass. The expression level was 51 % of the total with a relative concentration of 390 ng/µL.
The table illustrates the protein recovery as determined with the 2100 Bioanalyzer. Less than half of the POI in the total lysate was soluble and recovered in the lysate supernatant. As anticipated from the bead capacity, 45 mg were depleted from the lysate supernatant, and subsequently released by washes, SenP2 on-column cleavage and final imidazole elution. Together with the flow through, this adds up to 110 mg POI within the lysate supernatant.

Application note: 5990-6153EN

Protein Purification

Optimization of on-column cleavage



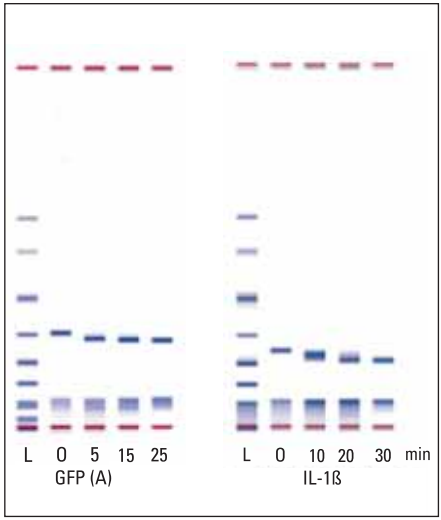
Sample	Size [kDa]	Rel. Conc. [ng/µL]	% Total
Unoptimized on-column	71.9	310.2	38.3
cleavage	81.9	414.7	51.3
Optimized on-column	70.9	993.0	78.0
cleavage	83.2	11.4	0.9

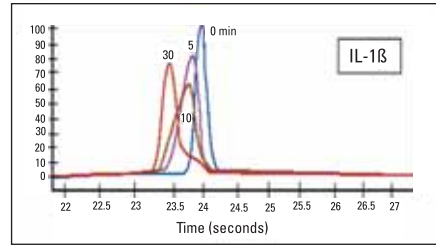
Kit: Protein 230 kit
Assay: Protein 230 assay
Application: Monitoring the protein of interest (POI) during purification in a fast and reliable manner is fundamental. Here, protein purification of a His6-Sumo3-tagged DNA-binding protein was performed with Immobilized Metal Affinity Chromatography (IMAC). The POI was N-terminally fused to His6-Sumo3-tag, bound to Ni beads, and released from the tag and beads by treatment with His-tagged SenP2 protease. Although the protease was used in excess for on-column cleavage, most of the protein was still found to be fused to its tag. Therefore, the on-column cleavage step was optimized by determining the optimal ratio of protein-to-beads-to-protease by monitoring the percentage of cleaved protein versus its tagged precursor with the Protein 230 assay. The molecular weight difference of about 10 kDa is sufficient to achieve a baseline separation between the tagged and tagless protein. As illustrated in the blue electropherogram, almost all tagged protein can be cleaved under the optimized conditions.

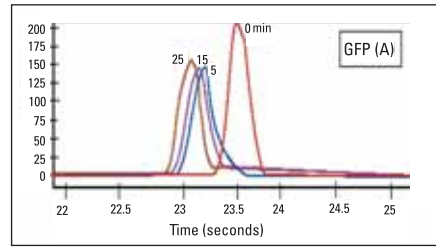
Application note: 5990-6153EN

Protein purification

Enzymatic removal of His-tags from recombinant proteins







Kit: Protein 200 Plus kit*
Assay: Protein 200 Plus assay*
Application: For some applications, it might be necessary to remove the His-tag after the protein purification because of its effects on enzymatic activity or protein structure. Here the TAGZyme system (Qiagen) was used to remove the N-terminal His-tag from two different proteins, a GFP variant and a recombinant Interleukin 1β. Samples were taken at different time points to study the kinetics of the enzymatic cleavage. The dipeptide cleavage can be detected by a size shift on the gel-like images and the electropherograms. The fast analysis with the bioanalyzer allows multiple kinetic studies to done in one day instead of waiting until the next day for the results from SDS-PAGE analysis.

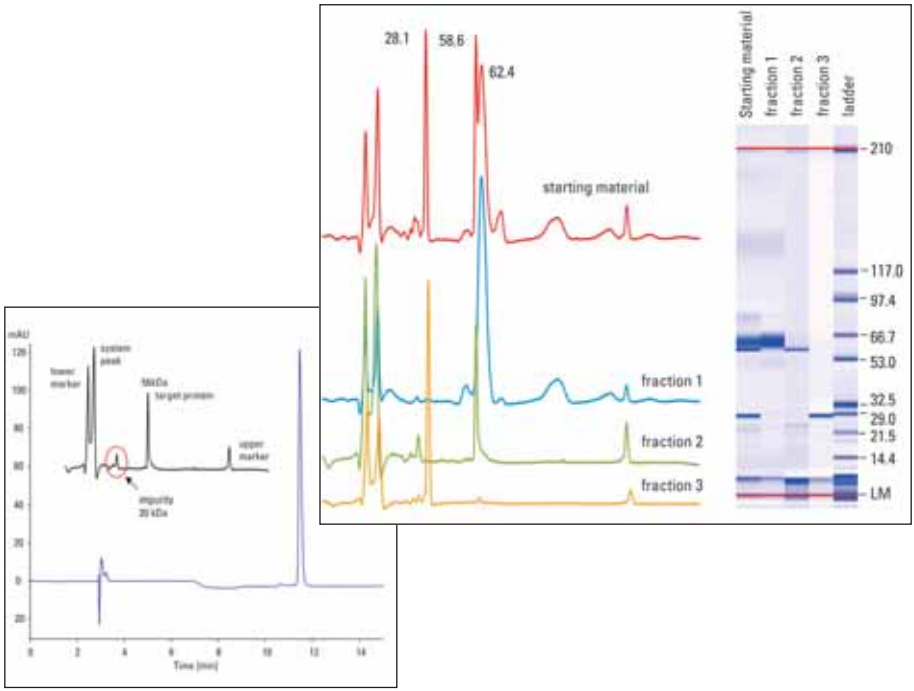
Poster presented at ABRF Conference, March 2002 by
F. Schäfer, K. Steinert, C. Feckler, J. Drees, and J. Ribbe, QIAGEN GmbH, Hilden, Germany

Application note: 5988-8144 EN

* replaced with Protein 230 kit and assay

Protein purification

Complementing RP-HPLC protein purification



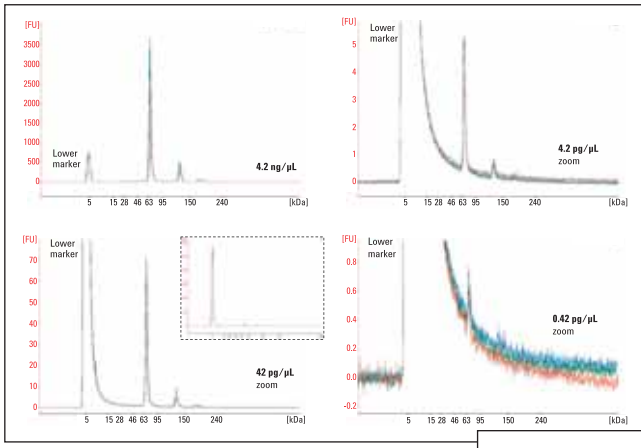
Kit: Protein 200 Plus kit*
Assay: Protein 200 Plus assay*
Application: Protein purification and characterization was carried out facilitating an 1100 Series purification system for reverse phase HPLC assisted by the 2100 Bioanalyzer. The final polishing of a 56 kDa protein by RP HPLC from a pre-purified sample (starting material, right: red electropherogram and gel) and the analysis of three HPLC-fractions containing the major components are shown (fractions 1-3). No impurity is visible by RP HPLC reanalysis (left chromatogram, fraction 2) of the fraction containing the target protein. However, because the 2100 Bioanalyzer is an orthogonal technique compared to reverse phase HPLC a 20 kDa protein could be found as an impurity (see insert). The reverse phase HPLC purification leads to a purity of only 76 % for the protein of interest and the 2100 Bioanalyzer reveals the necessity of further purification.

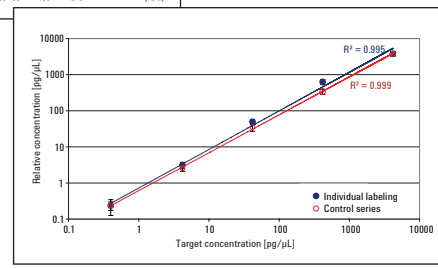
Application note: 5988-8630EN

* replaced with Protein 230 kit and assay

High sensitivity protein detection

Detection of low protein amounts



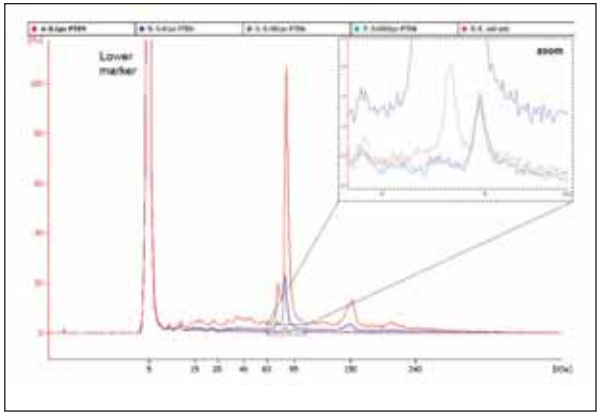


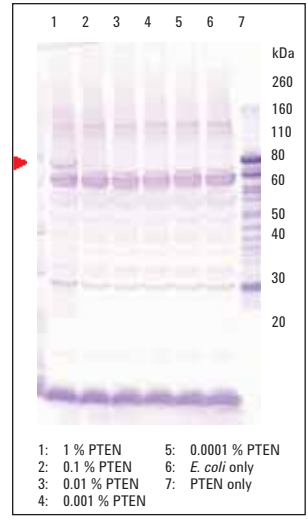
Kit: High Sensitivity Protein 250 kit
Assay: High Sensitivity Protein 250 assay
Application: For highest sensitivity, SDS-PAGE gels are commonly silver stained using a laborious procedure with low reproducibility and insufficient quantification capabilities. The High Sensitivity Protein 250 kit is a superior alternative to silver staining protocols due to reproducible and fast staining, automated separation and data analysis. It overcomes the critical limitations of traditional silver staining of SDS-PAGE gels by providing high sensitivity and a linear dynamic quantitation range of four orders of magnitude combined with excellent reproducibility. The kit analyzes proteins from 10 to 250 kDa down to an on chip concentration of 1 pg/µL. It is based on the detection of fluorescently labeled proteins that are separated by on-chip electrophoresis.

Technical note: 5989-8940EN

High sensitivity protein detection

Highly specific and sensitive alternative to Western blotting



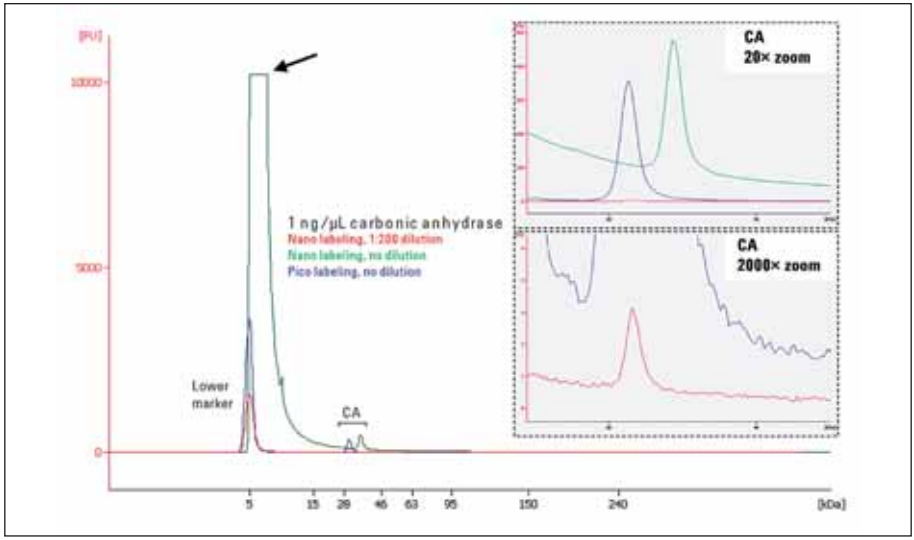


Kit: High Sensitivity Protein 250 kit
Assay: High Sensitivity Protein 250 assay
Application: A targeted protein analysis employing immunoprecipitation in combination with the High Sensitivity Protein 250 assay (IP/HSP250 method) was evaluated. Sensitivity and specificity of the IP/HSP250 method were investigated using *E. coli* lysate spiked with GST-tagged phosphatase and tensin homolog (PTEN). Samples were first fluorescently labeled, then immunoprecipitated, and finally the complexes formed were directly eluted from magnetic Protein A beads and analyzed with the High Sensitivity Protein 250 assay. Electropherograms of samples with 0.1 % to 0.0001 % PTEN and a negative control are shown. The zoom shows the main peak of 0.001 % PTEN. The limit of detection was determined to be 0.001 % or 100 pg PTEN in 10 µg *E. coli* lysate. For comparison, Western blots were performed. The PTEN blots showed a high non-specific background in all lanes, due to the secondary antibody. A specific band was observed only at 1 % or 100 ng PTEN in 10 µg *E. coli* lysate.
Thus, the IP/HSP250 method showed both higher sensitivity and specificity than the Western blot, resulting in a 1,000 fold lower limit of detection for PTEN.

Application note: 5990-4097EN

High sensitivity protein detection

Pico labeling protocol for samples below 1 ng/µL protein

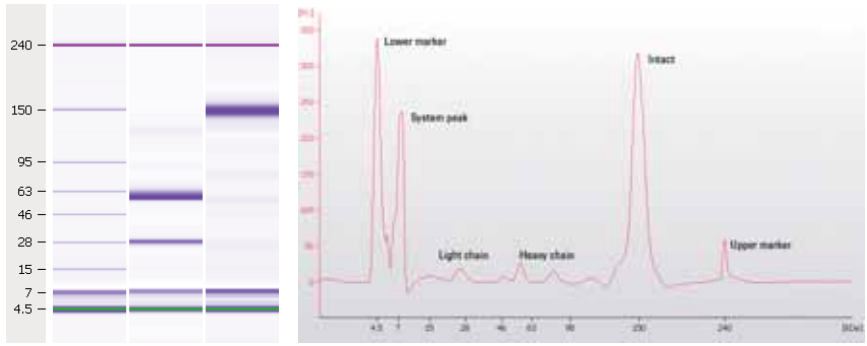


Kit: High Sensitivity Protein 250 kit
Assay: High Sensitivity Protein 250 assay
Application: The High Sensitivity Protein 250 assay for the 2100 Bioanalyzer allows detection of fluorescently-labeled proteins down to 1 pg/µL on-chip thus offering a sensitivity superior to silver-stained SDS-PAGE. However, the standard fluorescent labeling (Nano) method requires a minimum total protein concentration of 1 ng/µL in the initial sample. The alternative Pico labeling protocol extends the applicability to initial protein samples with concentrations from 1 ng/µL down to 10 pg/µL. The two labeling protocols were compared analyzing 1 ng/µL carbonic anhydrase (CA) labeled according to the Nano (red) and the Pico (blue) labeling protocol. When CA labeled with the Nano protocol but without the 200-fold dilution (green) was analyzed, the lower marker peak exceeded the measuring range of the 2100 Bioanalyzer. If the labeling reaction is performed with the Pico labeling protocol using 100-fold less fluorescent dye, no dilution of the labeled sample is necessary. This allows analyzing highly diluted proteins, such as secreted proteins in culture media, or the analysis of particularly small amounts of protein derived from laser micro dissections.

Application note: 5990-3703EN

Antibody analysis

Analysis of antibodies under reducing and non-reducing conditions

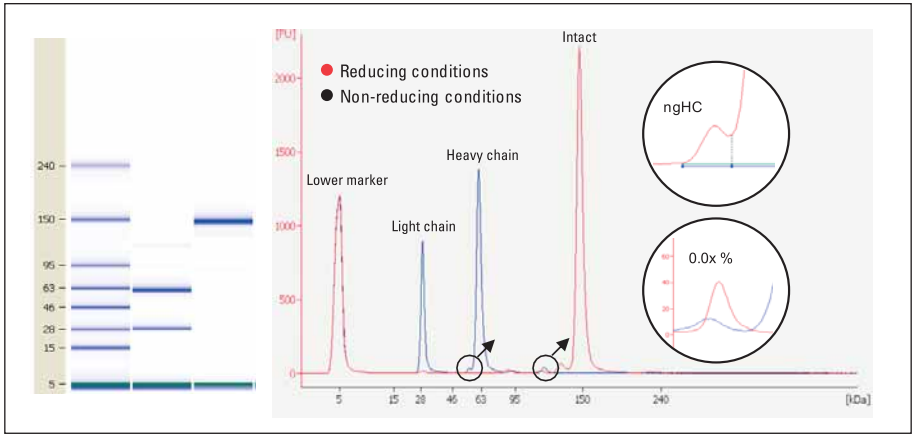


Kit: Protein 230 kit
Assay: Protein 230 assay
Application: The Protein 230 kit allows simultaneous analysis of antibodies under both conditions reduced and non-reduced on the same chip. Analyzing both in one experiment is not possible using SDS-PAGE, as the reducing agent will diffuse within the gel and will also reduce other samples. The Protein 230 assay sensitivity is in the same range as non-colloidal Coomassie staining. A systempeak (SP) as well as upper (UM) and lower (LM) marker are indicated. Under non-reducing conditions the intact antibody (size 149 kDa) represents 83 % of the total detectable protein. Besides other contaminants the light (lc) and heavy chains (hc) as well as half-antibodies are detected and characterized in size and quantity. Under reducing conditions light (28.2 kDa; 31 % of total) and heavy chain (58.2 kDa; 62 % of total) detection is dominant while higher aggregates are also detected.

Demo data file from P230 assay (Expert revision B.02.06 software)

Antibody analysis

High sensitivity analysis of antibodies

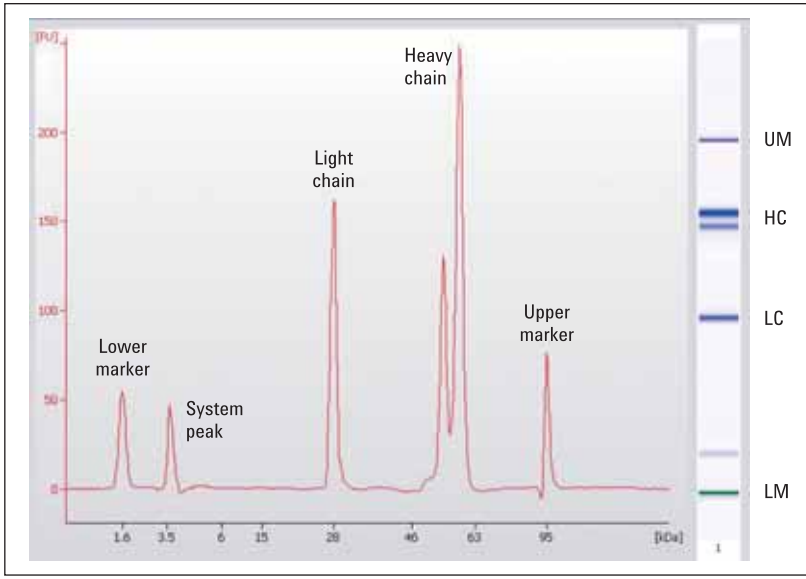


Kit: High Sensitivity Protein 250 kit
Assay: High Sensitivity Protein 250 assay
Application: The Protein 250 kit provides analysis of antibodies at highest sensitivity which is equal or better than silver staining sensitivity in SDS-PAGE. The high dynamic range of the Protein 250 assay facilitates impurity detection down to the pg/µL range and low percentage according to regulatory requirements. Both conditions, reduced (blue) and non-reduced (red), can be analyzed parallel on the same chip.
For this preparation the intact antibody (size 147.5 kDa) represents 92.62 % of the total detectable protein under non-reducing conditions. Besides other contaminants the light (lc, 0.74 %) and heavy chains (hc, 0.16 %) as well as half-antibodies (89 kDa; 1.05 %) are precisely quantified in relation of the total protein. Under reducing conditions light (28.9 kDa; 35.14 % of total) and heavy chain (61.2 kDa; 61.51 % of total) detection is dominant while higher aggregates can be identified. For this example antibody non-glycosylated heavy chain fragments (ngHC, 1.05 % of total) could be separated.

Demo data file from P250 assay (Expert revision B.02.06 software)

Antibody analysis

QA/QC of IgG under reducing conditions

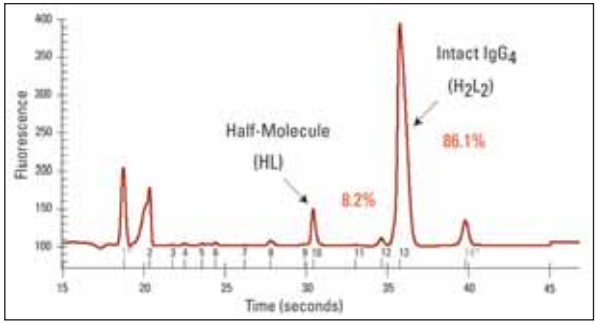


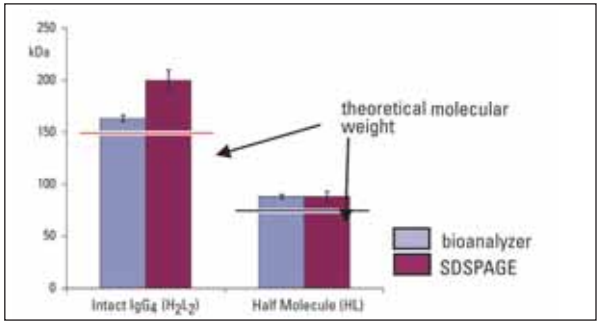
Kit: Protein 80 kit
Assay: Protein 80 assay
Application: The Protein 80 kit and the 2100 Bioanalyzer can be used for on-chip protein electrophoresis of IgG under reducing conditions. Due to the reduction of the disulfide bonds, in this case with DTT, the IgG is separated in its light and heavy chains. The electropherogram and the gel-like image show three main peaks/bands; the light chain, and two heavy chains, with and without glycosylation. No degradation was detected. This type of analysis is required for protein QA/QC to monitor protein stability, e.g. degradation and integrity, and to determine protein contaminations.

Data not published

Antibody analysis

Quantitation of the half-antibody content in IgG_4 preparations





Kit: Protein 200 Plus kit*
Assay: Protein 200 Plus assay*
Application: In the given host cell line for antibody production usually up to 30 % of IgG_4 is secreted as half molecule (half antibody). The half-molecule has only a single disulfide bond between the heavy and light chains, the inter-heavy chain disulfide bonds are absent. The protein assay allows the half-antibody content in IgG_4 preparations to be determined automatically. In addition, the sizing provided by the 2100 Bioanalyzer compares very well to the theoretical size and is superior to SDS-PAGE in terms of accuracy and reproducibility.

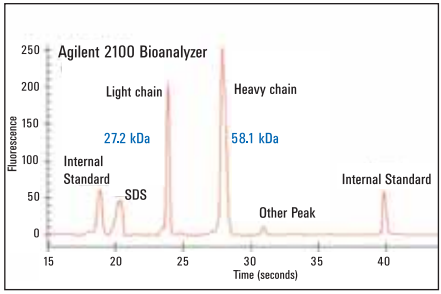
Poster presented at WCBP Conference, January 27-30, 2002 by E. Vasilyeva, H. Fajardo, P. Bove, F. Brown and M. Kretschmer, BIOGEN, Cambridge, MA, USA

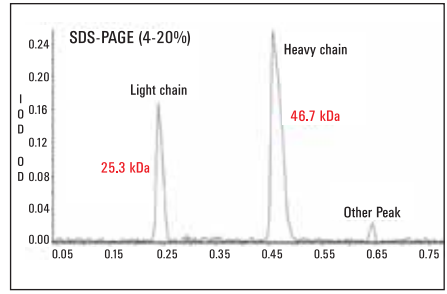
Data not published

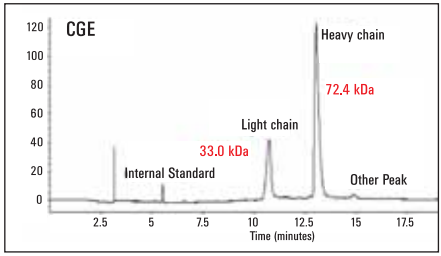
* replaced with Protein 230 kit and assay

Antibody analysis

Comparison of SDS-PAGE, CGE and 2100 Bioanalyzer for humanized monoclonal antibody analysis







Kit: Protein 200 Plus kit*
Assay: Protein 200 Plus assay*
Application: The analysis of a humanized monoclonal antibody under reducing condition was compared using 3 different techniques, the 2100 Bioanalyzer, 4-20 % SDS-PAGE, stained with Coomassie, and capillary gel electrophoresis. All 3 techniques result in a similar separation pattern showing the light and the heavy chain of the antibody. In addition, the determined sizes of the light and heavy chain were comparable for all 3 techniques and compared well to the molecular weights determined by MALDI-TOF (light chain: 23762 Da, heavy chain: 51003 Da). However, the 2100 Bioanalyzer provides significant time saving compared to the other techniques.

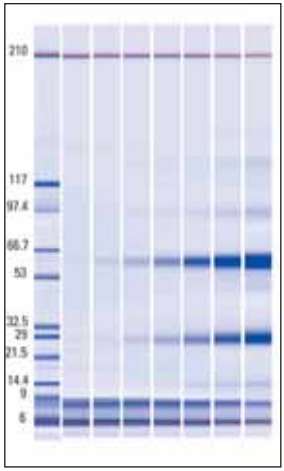
Poster presented at WCBP Conference, January 2002 by
S.H. Bowen, M. Chan, P. McGeehan, J. Smith, L. Inderdass, R. Strouse, M. Schenerman
MedImmune Inc., Gaithersburg, MD, USA

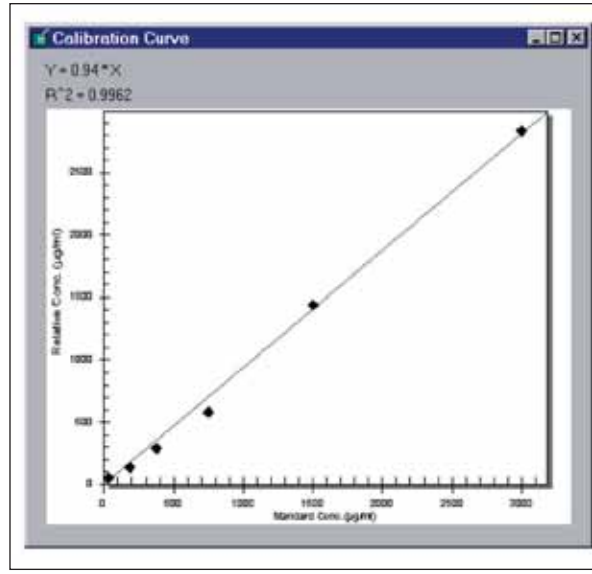
Data not published

* replaced with Protein 230 kit and assay

Antibody analysis

Absolute quantitation of IgG





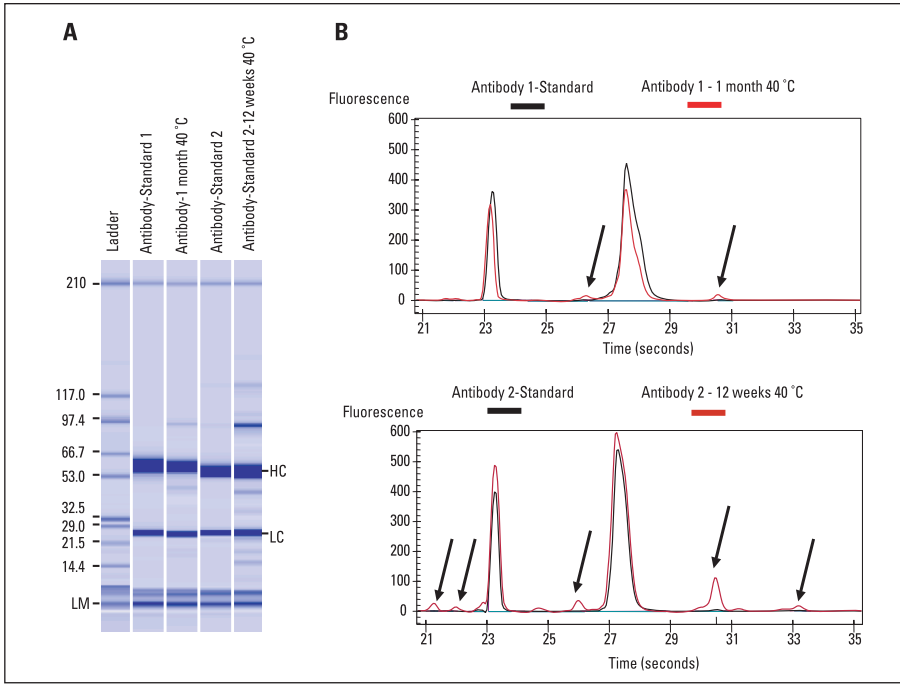
Kit: Protein 200 Plus kit*
Assay: Protein 200 Plus assay*
Application: The calibration feature of the software allows determination of the absolute antibody concentration in comparison to user defined standards with known concentration, accurate determination of IgG concentrations and carrying out batch comparison during antibody QA/QC.

Application notes: 5988-4021EN and 5988-6576EN

* replaced with Protein 230 kit and assay

Antibody analysis

Quality control of stressed antibodies



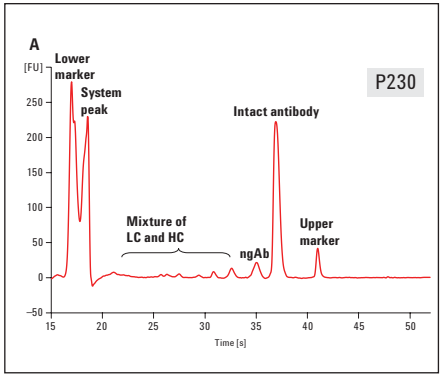
Kit: Protein 200 Plus kit*
Assay: Protein 200 Plus assay*
Application: A quality control step in pharmaceutical QA/QC departments is to trigger typical degradation and aggregation patterns for a specific antibody. The given samples from heat stress stability studies show expected protein byproducts after aging at elevated temperatures. The content of heavy and light chain, representing the intact antibody, is reduced by 5 % or 13 % within 1 month or respectively 12 weeks. Excellent reproducibility in the range from 0.6 to 1.7 % CV for this quantification was achieved in a validation study with three different users and two bioanalyzer instruments over several days.

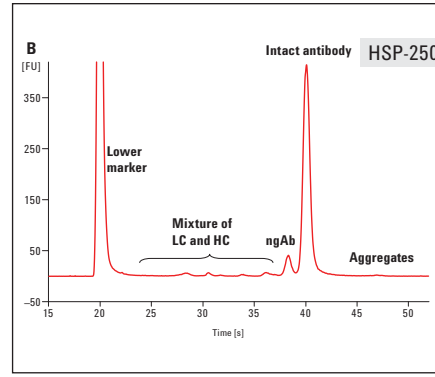
Application note: 5988-9648EN

* replaced with Protein 230 kit and assay

Antibody analysis

Analysis of IgG2 under non-reducing conditions



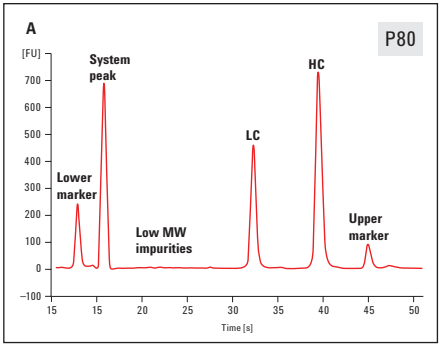


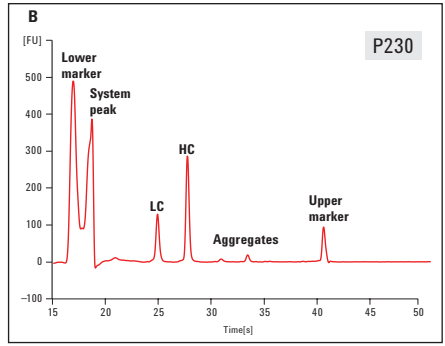
Kit: Protein 230 and High Sensitivity Protein 250 kit
Assay: Protein 230 and High Sensitivity Protein 250 assay
Application: Human myeloma IgG2 was analyzed with the 2100 Bioanalyzer and the Protein 230 (P230) and High Sensitivity Protein 250 (HSP-250) assay under non-reducing conditions. One representative electropherogram per assay is shown. The intact IgG2 antibody is detected at 156.6 kDa, which is in close agreement with its theoretical molecular mass of about 150 kDa. A unique feature of the HSP-250 assay is the size and concentration measurement beyond the size range of the ladder, that is, 250 kDa. Therefore, high molecular weight aggregates or impurities above 250 kDa are sized and quantified as well. The P230 and HSP-250 assays clearly resolve light chain (LC), heavy chain (HC) and a mixture of LC and HC peaks including the non-glycosylated form of IgG2 (ngAb).

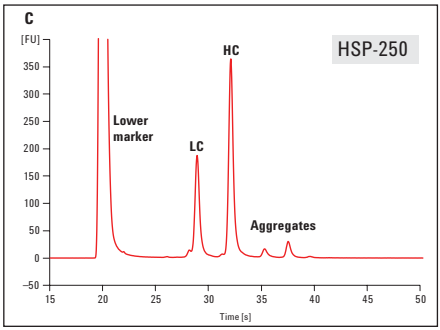
Application note: 5990-5283EN

Antibody analysis

IgG2 analysis under reducing conditions





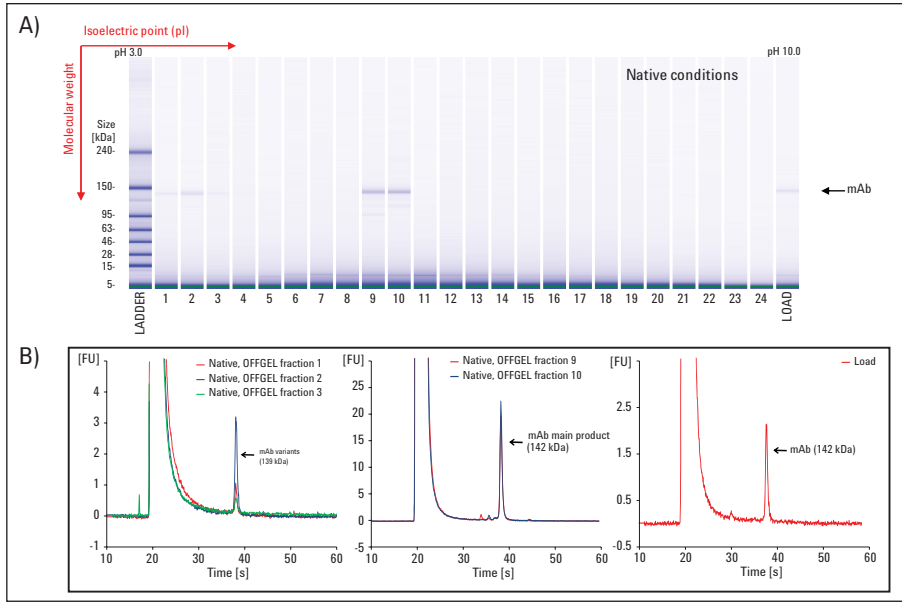


Kit: Protein 80, 230 and High Sensitivity Protein 250 kit
Assay: Protein 80, 230 and High Sensitivity Protein 250 assay
Application: Human myeloma IgG2 was analyzed with the 2100 Bioanalyzer and the Protein 80 (P80), the Protein 230 (P230) and the High Sensitivity Protein 250 (HSP-250) assay in the presence of dithiothreitol (DTT) as reducing agent. One representative electropherogram per assay is shown. Under reducing conditions, the IgG2 light chain (LC) and heavy chain (HC) are well resolved with all three 2100 Bioanalyzer protein assays. Aggregates of higher molecular weight are observed with the P230 and HSP-250 assays whereas the P80 assay resolves low molecular weight (MW) impurities associated with the IgG2 sample.

Application note: 5990-5283EN

Antibody analysis

Monitoring antibody charge variants



Kit: High Sensitivity Protein 250 kit

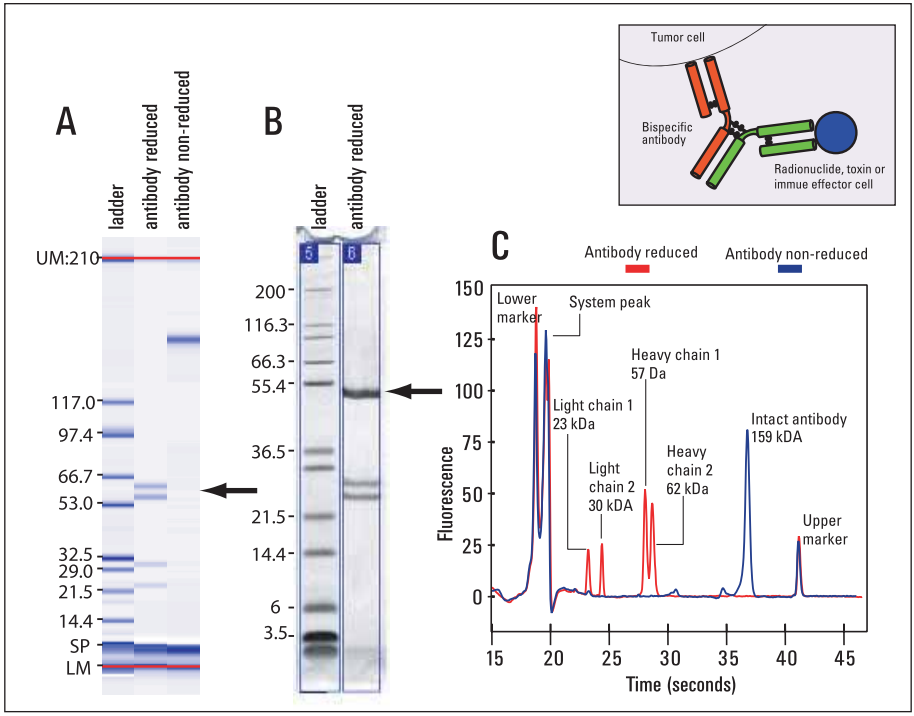
Assay: High Sensitivity Protein 250 assay

Application: During production and purification, antibodies can undergo modifications leading to charge heterogeneity, impacting stability, activity and causing immunologically adverse reactions. The investigation of monoclonal antibody (mAb) heterogeneity is critical for quality control, preferably combining charge and size analysis. In the first dimension, 3100 OFFGEL fractionation, an isoelectric focusing technique delivering fractions in solution, was performed and in the second dimension, the fractions were size separated using the High Sensitivity Protein 250 assay. Under native conditions various mAb charge variants exhibiting acidic pI values were detected (A). The electropherogram overlays show the acidic charge variants, the main product and the initial sample load (B). The charge variants differ from the main mAb by a maximum of 4 kDa and represent only 10 % of the total mAb preparation. Therefore, only the combination of both methods allows the detection of the mAb main product and its charge variants.

Application note: 5990-6521EN

Antibody analysis

Separation of bispecific antibody chains



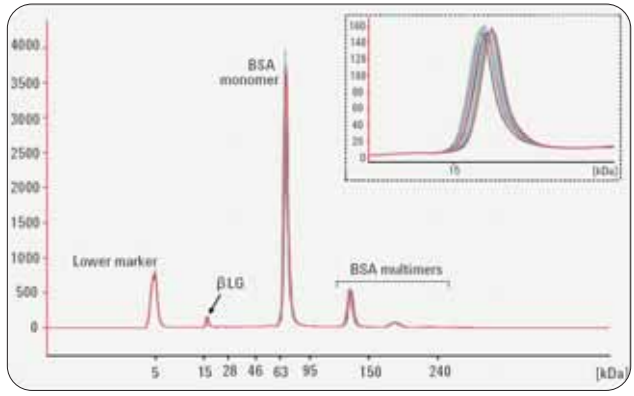
Kit: Protein 200 Plus kit*
Assay: Protein 200 Plus assay*
Application: In general, antibodies are biopharmaceuticals of great interest. Especially bispecific antibodies often require high resolution to allow analysis of both sets of chains (2100 Bioanalyzer: A, gel like view, resolved heavy chains; C electropherogram). A labor intensive SDS-PAGE could not resolve the heavy chains (B, marked by an arrow) in the given sample. In contrast, the 2100 Bioanalyzer is a superior tool for antibody quality control since it is a convenient, fast and easy to standardize method which additionally enables quantitative analysis.

Application note: 5988-9651EN

* replaced with Protein 230 kit and assay

Protein quantification

Quantification of low protein amounts with an internal standard



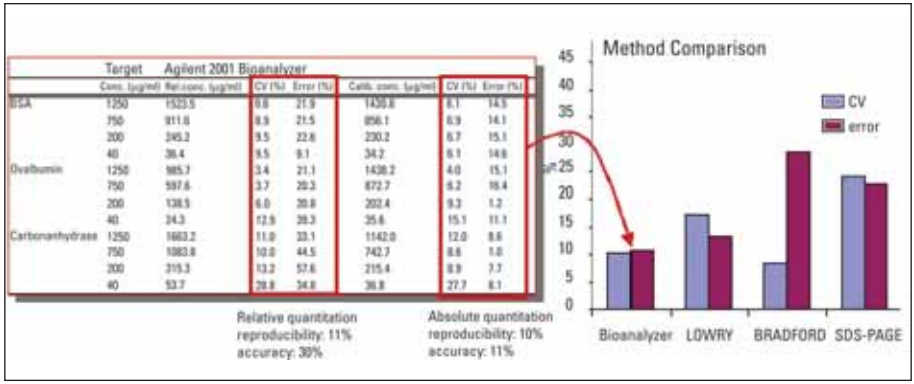
Protein		Rel. Conc. [pg/µL]	% Total
βLG	Average	184	3.3
	% CV	3.3	1.5
BSA	Average	4191	75.7
monomer	% CV	3.1	0.3

Kit: High Sensitivity Protein 250 kit
Assay: High Sensitivity Protein 250 assay
Application: The High Sensitivity Protein 250 assay is based on the detection of fluorescently labeled proteins that are separated by on-chip electrophoresis. The quantification and sizing of sample peaks is done relative to a ladder as an external standard on the same chip, in contrast to the Agilent Protein 80 and Protein 230 assays where the quantification is based on internal standards. With the High Sensitivity Protein 250 kit it is also possible to do quantification based on the addition of a suitable internal standard. In this example a small standard protein, ß-Lactoglobulin (ßLG, 18.4 kDa), was used. ßLG was spiked into a BSA solution prior to the labeling reaction and the analysis was performed according to the standard protocol. The electropherogram shows an overlay of 10 runs and a zoom on the ßLG peak in the insert. The quantification data for the ßLG and BSA monomer is summarized in the table. Quantification reproducibility is improved using an internal standard, because well-to-well variations for sample injection and matrix influences during staining are excluded.

Application note: 5989-8941EN

Protein quantification

Absolute protein quantitation



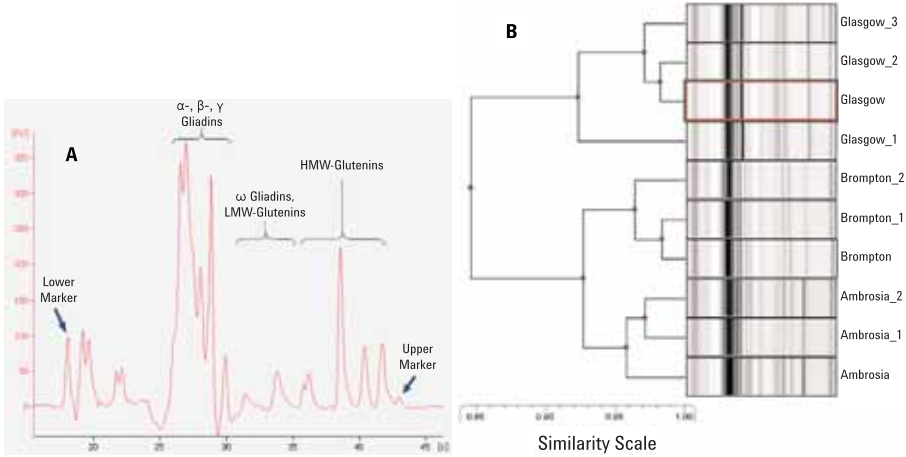
Kit: Protein 200 Plus kit*
Assay: Protein 200 Plus assay*
Application: A comparative analysis of different techniques used for absolute protein quantitation was performed analyzing 3 different proteins (CA, BSA, OV) in 4 different concentrations (40 – 1250 µg/mL). The same samples were quantitated using the 2100 Bioanalyzer, two commonly used total protein quantitation assays, Lowry and Bradford, and SDS-PAGE, stained with Coomassie. The relative standard deviation (CV) and the error compared to the target concentration were determined. A comparison shows that the CV and error for the 2100 Bioanalyzer are better than for the SDS-PAGE by a factor of 2. This data demonstrates that the 2100 Bioanalyzer is a viable alternative for protein quantitation. It allows the quantitation of individual proteins and simultanous determination of protein purity and size.

Application notes: 5988-4021EN and 5988-6576EN

* replaced with Protein 230 kit and assay

Food analysis

Rapid wheat varietal identification

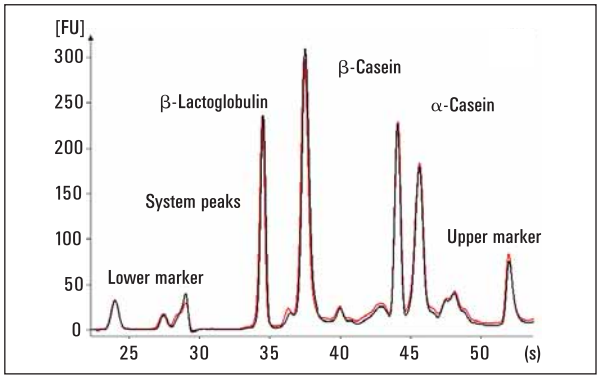


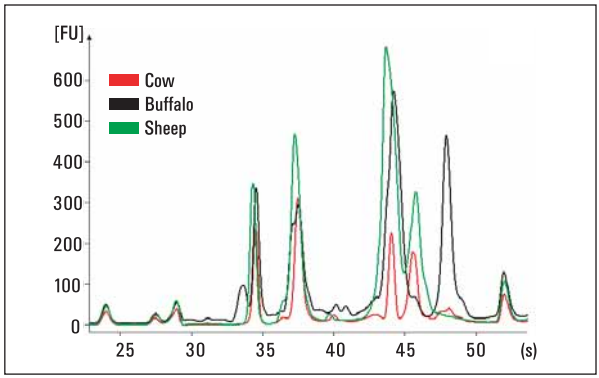
Kit: Protein 230 kit
Assay: Protein 230 assay
Application: Total wheat proteins (including high molecular weight glutenins, HMW) were extracted from individual grains. The extract samples were separated on the 2100 Bioanalyzer. The Protein 230 assay produced well-resolved protein profiles, suitable for varietal discrimination (A). Electropherogram profiles were processed using the Phoretix 1D Advanced and 1D Database (Nonlinear Dynamics) software for pattern-matching purposes. Replicates of three different wheat varieties can be correctly grouped in a dendrogram (B). The study has demonstrated that using the 2100 Bioanalyzer with the Phoretix system offers a standardized, objective method for rapid varietal discrimination. The ease of use and short analysis times of less than one hour from sampling to 2100 Bioanalyzer result makes it most suitable for mill intake use.

Application note: 5989-7735EN

Food analysis

Bovine milk analysis





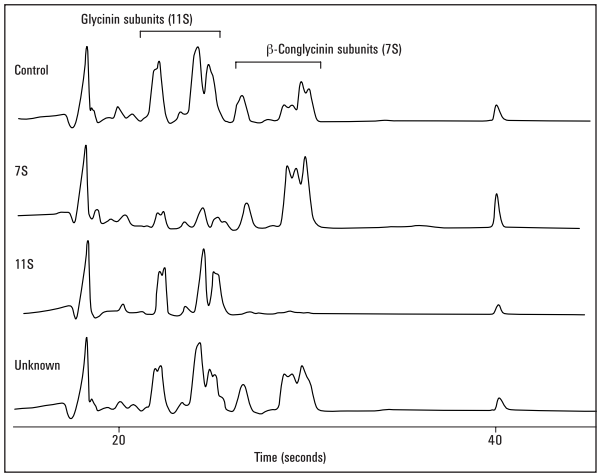
Kit: Protein 50 kit*
Assay: Protein 50 assay*
Application: The Protein 50 is suitable for analysis of diary products such as milk.
The kit delivers an excellent reproducibility, as shown in this example (A, bovine milk diluted 1:10). Here the main protein fractions can be identified running the individual purified proteins for comparison (not shown). The overlay of the electropherograms from two separate runs under reducing conditions demonstrates the high reproducibility of the assay. Furthermore, milk from different animals could be distinguished based on their protein pattern (B) which facilitates a fast incoming inspection in routine labs.

Data not published

* replaced with Protein 80 kit and assay

Food analysis

Protein pattern of different transgenic seedlines



SeedLine	Extracted protein level µg/mL	7S/11S Ratio
Control	14,000	0.39±0.004(n=5)
7S	5,200	3.4
11S	14,000	0.04
Unknows	13,000	0.72±0.1(n=20)

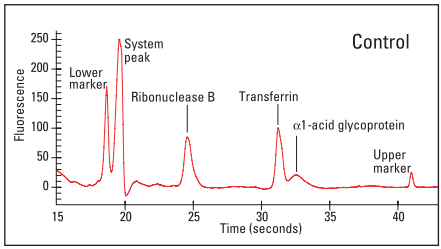
Kit: Protein 200 Plus kit*
Assay: Protein 200 Plus assay*
Application: Determination of protein size and concentration with sufficient accuracy and precision allows the highly efficient characterization of transgenic seed lines. Expressed protein was available after grinding, extraction of seeds and dilution with buffer. Electropherograms were evaluated by integration of regions specific for 7S or 11S seed storage proteins.
The elevated ratio of 7S/11S for the analyzed unknown line shows significant changes in the expression profile in comparison with the control.

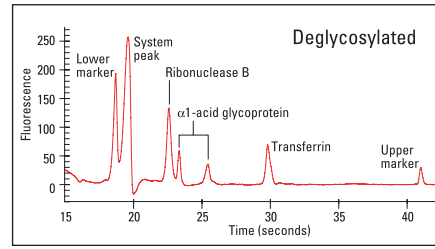
Application note: 5988-9441EN

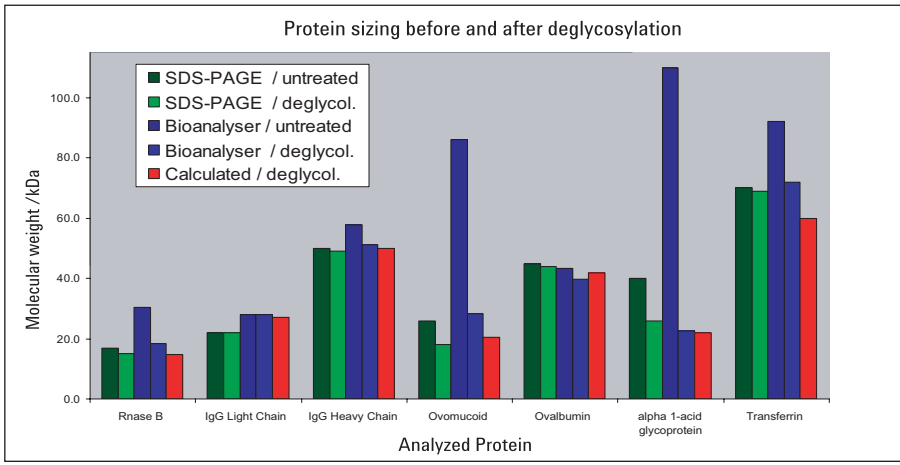
* replaced with Protein 230 kit and assay

Protein – others

Glycoprotein sizing







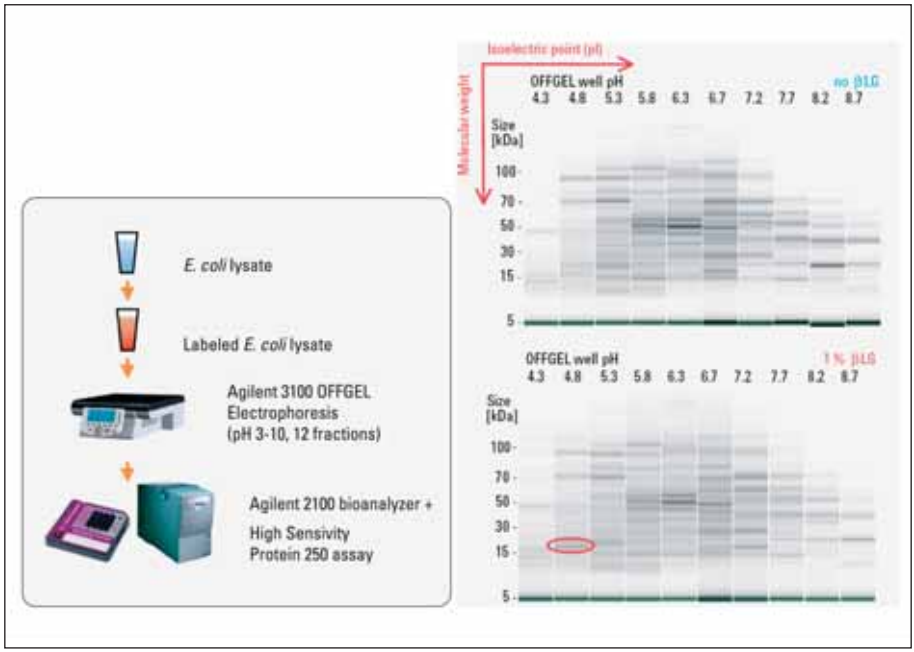
Kit: Protein 200 Plus kit*
Assay: Protein 200 Plus assay*
Application: Due to large carbohydrate moieties glycosylated proteins can differ in amount of incorporated SDS and shape of the protein/SDS-complex from non-glycosylated proteins. This may lead to different migration times in SDS-PAGE, as well as in the Protein 200 Plus assay* run on the 2100 Bioanalyzer. The data compare deglycosylation of a mixture of three proteins (electropherogram on the left) with a commercial N-Glycosidase F Deglycosylation kit. Sizing experiments comparing glycosylated and non glycosylated states for additional proteins are compared and summarized on the right. Such an approach avoids misinterpretation of sizing due to glycosylation and allows detection of a posttranslational modification of unknown proteins.

Application note: 5989-0332EN

* replaced with Protein 230 kit and assay

Protein – others

OFFGEL electrophoresis combined with high-sensitivity on-chip protein detection

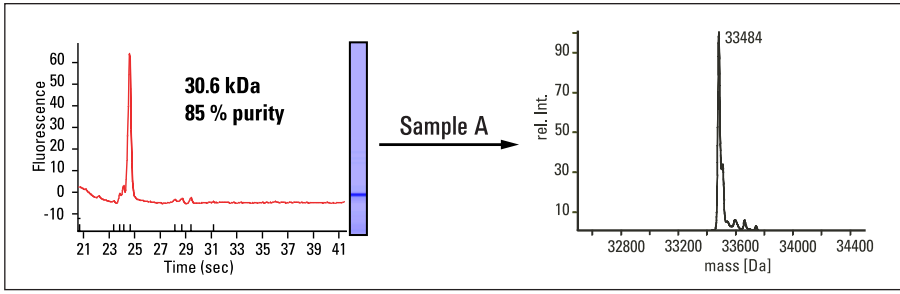


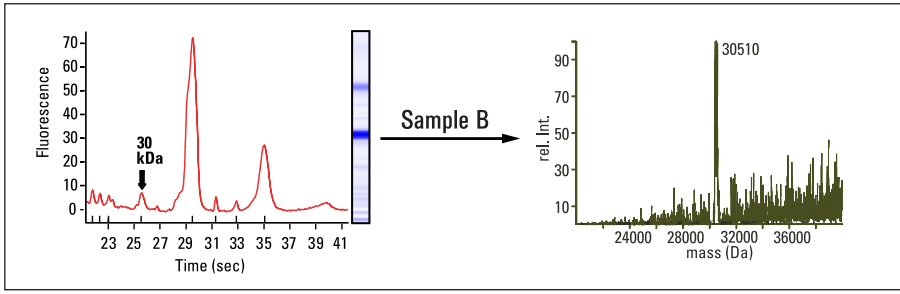
Kit: Protein 250 kit
Assay: Protein 250 assay
Application: Two-dimensional gel electrophoresis (2D-GE) is a tedious and time-consuming procedure. The combination of fractionation with the Agilent 3100 OFFGEL fractionator based on isoelectric point (pI), and high-sensitivity on-chip electrophoresis with the 2100 Bioanalyzer performs an analytical 2D-GE-type analysis with excellent sensitivity and reproducibility. The ease of use is significantly improved compared with the traditional 2D-GE method. The OFFGEL fractionation method takes advantage of the impressive resolving power of immobilized pH gradient-based isoelectric focusing (IPG-IEF) and delivers the sample in liquid phase. The High Sensitivity Protein 250 assay is based on the detection of fluorescently labeled proteins that are electrophoretically separated, allowing separation of proteins from 10 to 250 kDa, with a sensitivity equivalent to or better than silver staining, and delivers a linear dynamic range across four orders of magnitude.

Application note: 5989-8419EN

Protein – others

Protein quality control prior to MS-analysis





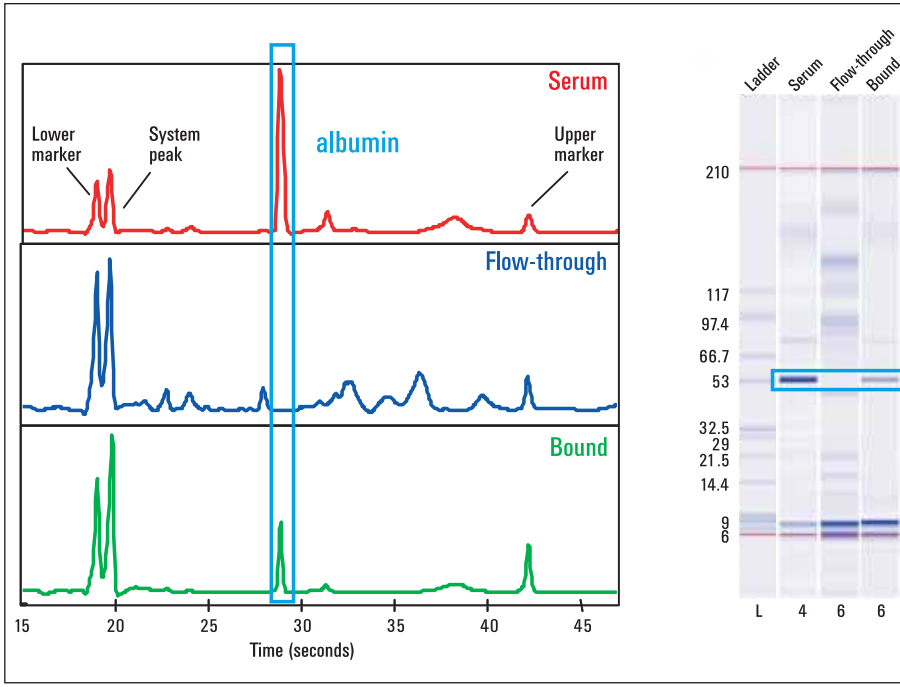
Kit: Protein 200 Plus kit*
Assay: Protein 200 Plus assay*
Application: By applying soft ionization methods like MALDI or ESI mass spectroscopy (MS), mass information from proteins up to 300 kDa can be obtained. However, proper sample preparation is an important precondition. Concentration, purity and assumed size are valuable ex ante information usually given by biochemists to MS-analysis services. Two different examples for proteins analyzed by an LC/MS-method (right panel) with good results for sample A and discrepancies for sample B are shown. An analysis of the samples with the Protein 200 Plus assay* (left panel) showed an impure protein preparation for sample B. Here, two major peaks at higher masses (66 and 132 kDa) potentially caused by aggregates were encountered. The protein of interest (30 kDa) yielded high noise background in the MS. A quality check of the sample with the Protein 200 Plus assay, therefore, may avoid an unproductive MS analysis or data evaluation.

Application note: 5989-0771EN

* replaced with Protein 230 kit and assay

Protein – others

Depletion of high abundant proteins from blood samples



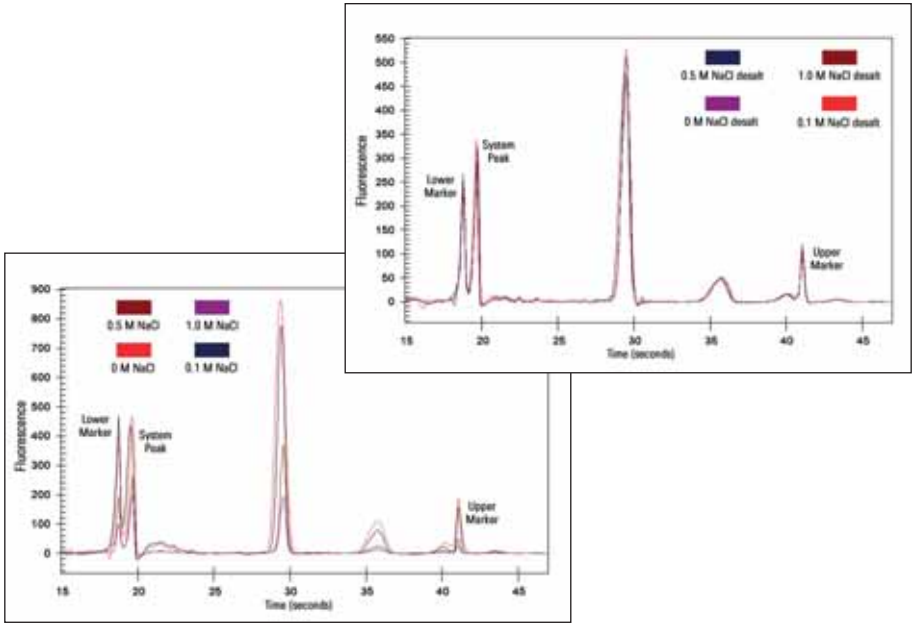
Kit: Protein 200 Plus kit*
Assay: Protein 200 Plus assay*
Application: Depletion of high abundant proteins in human blood plasma was facilitated by the Agilent Multiple Affinity Removal System. Unprocessed serum, the flow through (i.e. the immunodepleted serum) and the bound proteins after specific elution were analyzed on the 2100 Bioanalyzer. Equivalent amounts were analyzed and resulting electropherograms (left) and gel like images (right) show in comparison the completeness of separation. The 2100 Bioanalyzer, in combination with the Protein 200 Plus kit*, proves to be an excellent method for evaluation of serum processed with albumin removal kits. The system offers a rapid and accurate method to detect proteins both quantitatively and qualitatively.

Data not published

* replaced with Protein 230 kit and assay

Protein – others

Increased sensitivity by desalting protein samples



Kit: Protein 200 Plus kit*
Assay: Protein 200 Plus assay*
Application: Protein purification steps, such as ion exchange chromatography, often implicate high salt concentrations. Nevertheless, quantitation of these samples is effective since the upper marker serves as internal protein standard and is subjected to the same conditions. However, under high salt conditions lower amounts of protein are injected into the microfluidic channels for analysis. Therefore, the sensitivity can be increased by usage of convenient desalting spin columns. Comparably higher and homogeneous peak intensities are obtained while the recovery after such treatment is good.

Application note: 5989-0228EN

* replaced with Protein 230 kit and assay

Literature

Application notes and other publications

To download an application note or to find other literature on the 2100 Bioanalyzer, visit the literature library section at: **www.agilent.com/genomics/literature**

Cell Applications	Publication number
Protein Expression	
Detection of antibody-stained cell surface and intracellular protein targets with the Agilent 2100 Bioanalyzer	5988-4322EN
Detecting cell surface proteins with the Agilent 2100 Bioanalyzer by on-chip antibody staining	5988-7111EN
Flow cytometric analysis of a limited number of cells using the Agilent 2100 Bioanalyzer	5989-0746EN
A new method for the calculation of baculovirus titre using the Agilent 2100 Bioanalyzer and the flow cytometry kit	5989-1644EN
Cytometric analysis of upregulated functional gene expression in primary cells by on-chip staining	5989-2718EN
A new tool for routine testing of cellular protein expression: integration of cell staining and analysis of protein expression on a microfluidic chip-based system	5989-0021EN
Transfection Efficiency	
Monitoring transfection efficiency by green fluorescence protein (GFP) detection	5988-4320EN
Monitoring transfection efficiency in cells using an on-chip staining protocol	5988-7296EN
Detecting cell surface proteins with the Agilent 2100 Bioanalyzer by on-chip antibody staining	5988-7111EN
Flow cytometric analysis of human primary cells using the Agilent 2100 Bioanalyzer and on-chip staining	5988-8154EN
Apoptosis	
A fast protocol for apoptosis detection by annexin V with the Agilent 2100 Bioanalyzer	5988-7297EN
Apoptosis detection by annexin V and active caspase-3 with the Agilent 2100 Bioanalyzer	5988-4319EN
Detection of apoptosis in primary cells by annexin v binding using the Agilent 2100 Bioanalyzer	5989-2934EN
Measuring multiple apoptosis parameters with the Agilent 2100 Bioanalyzer	5988-8028EN

Gene Silencing

siRNA transfection optimization with the Agilent 2100 Bioanalyzer	5988-9872EN
Confirming gene silencing mechanism by pGFP/GFP22 – siRNA co-transfection	5989-0103EN

Others

Cell fluorescence assays on the Agilent 2100 Bioanalyzer – general use	5988-4323EN
Identification of red and white blood cells from whole blood samples using the Agilent 2100 Bioanalyzer	5989-7171EN

DNA Applications

	Publication number

Restriction digest

High precision restriction fragment sizing with DNA 12000 kit	5968-7501EN
Quantitative analysis of PCR fragments with DNA 7500 kit	5968-7496EN

PCR

Quantitative analysis of PCR fragments with DNA 7500 kit	5968-7496EN
High resolution DNA analysis with the DNA 500 and DNA 1000 kits	5988-3041EN
Optimizing real-time quantitative PCR experiments with the Agilent 2100 Bioanalyzer	5989-7730EN
Quantitative analysis of PCR fragments with DNA 7500 kit	5968-7496EN
Highly efficient multiplex PCR using novel reaction chemistry	5988-9342EN
Interference of SybrGreen in detecting PCR amplicons with the Agilent 2100 Bioanalyzer	5989-4458EN
Complete Automation of the Stratagene StrataPrep 96 PCR Purification Kit with the Agilent Bravo Automated Liquid Handling Platform and Agilent Automated Centrifuge	5990-3948EN

Gene expression

Quantitative end-point RT-PCR gene expression using DNA 7500 kit	5988-3674EN
Semiquantitative reverse transcription-polymerase chain reaction with the Agilent 2100 Bioanalyzer	5988-4556EN
DNA QC for oligonucleotide array CGH (aCGH) with the Agilent 2100 Bioanalyzer	5989-2487EN

Next-generation sequencing

Automation of Agencourt AMPure Purification Kit for the Purification of Next-Generation Sequencing Sample Preparation Reactions on the Agilent Bravo Automated Liquid Handling Platform	5990-4942EN
Improving sample quality for target enrichment and next-gen sequencing with the Agilent High Sensitivity DNA Kit and the Agilent SureSelect Target Enrichment Platform	5990-5008EN

Food

Development of meat speciation assays using the Agilent 2100 Bioanalyzer	5988-4069EN
Determination of PCR-RFLP profiles for fish species using the Agilent 2100 Bioanalyzer	5989-2982EN
Analysis of genetically modified soya using the Agilent 2100 Bioanalyzer	5988-4070EN
Nested multiplex PCR for the determination of DNA from genetically modified corn and soy beans using the Agilent 2100 Bioanalyzer	5989-0124EN
Use of the Agilent 2100 Bioanalyzer for basmati rice authenticity testing	5989-6836EN

GMO

Detecting genetically modified organisms with the Agilent 2100 Bioanalyzer	5988-4847EN
Analysis of genetically modified soya using the Agilent 2100 Bioanalyzer	5988-4070EN
Nested multiplex PCR for the determination of DNA from genetically modified corn and soy beans using the Agilent 2100 Bioanalyzer	5989-0124EN

Oncology

Sensitive detection of tumor cells in peripheral blood of carcinoma patients by a reverse transcription PCR method	5988-9341EN
Mutation detection for the K-ras and P16 genes	5989-0487EN
Measuring the METH-2 promoter hypermethylation and transcript downregulation in non-small cell lung carcinomas with the Agilent 2100 Bioanalyzer	5989-3514EN
Label-free analysis of microsatellite instability in colorectal carcinoma by on-chip electrophoresis	5989-2626EN
Agilent 2100 Bioanalyzer replaces gel electrophoresis in prostate cancer research	5988-1086EN

Clinical research

Microfluidic analysis of multiplex PCR products for the genotyping of Helicobacter pylori	5989-0078EN
Rapid detection of genomic duplications and deletions using the Agilent 2100 Bioanalyzer	5989-0192EN
Detection of a point mutation in the prothrombin gene with the Agilent 2100 Bioanalyzer	5989-4313EN

Forensic

Using the Agilent 2100 Bioanalyzer to optimize the PCR amplification of mitochondrial DNA sequences	5989-3107EN
Use of the Agilent 2100 Bioanalyzer and the DNA 500 in the analysis of PCR amplified mitochondrial DNA	5989-0985EN

Others

Analysis of Cy5-labeled cRNAs and cDNAs using the Agilent 2100 Bioanalyzer and the RNA 6000 kit	5980-0321EN
Assessing genomic DNA contaminations of total RNA isolated from kidney cells obtained by laser capture microdissection using the Agilent RNA 6000 Pico assay	5989-0991EN
Measuring multiple apoptosis parameters with the Agilent 2100 Bioanalyzer	5988-8028EN
siRNA transfection optimization with the Agilent 2100 Bioanalyzer	5988-9872EN

Technical

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New Series II DNA assays for the Agilent 2100 Bioanalyzer	5989-4652EN
Performance characteristics of the High Sensitivity DNA kit for the Agilent 2100 Bioanalyzer	5990-4417EN

RNA Applications

	Publication number

Total RNA

Analysis of total RNA using the RNA 6000 kit	5968-7493EN
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Isolation of high purity total cellular RNA from muscle tissues	5989-2312EN
Optimizing real-time quantitative PCR experiments with the Agilent 2100 Bioanalyzer	5989-7730EN
Quantitation comparison of total RNA using the Agilent 2100 Bioanalyzer, ribogreen analysis and UV spectrometry	5988-7650EN
Stringent RNA quality control using the Agilent 2100 Bioanalyzer	5989-1086EN
The total RNA story	5988-2281EN
Characterization of RNA quality using the RNA 6000 kit	5980-0472EN
Advancing the quality control methodology to assess isolated total RNA and generated fragmented cRNA	5988-9861EN
High-Purity RNA isolation from a wide range of plant species and tissue types	5989-2271EN
Automation of Stratagene Absolutely RNA 96 Microprep Kit with the Bravo Automated Liquid Handling Platform	5990-3558EN
Gene Expression Microarray Analysis of Archival FFPE Samples	5990-3917EN

Low amounts of RNA

Quality assurance of RNA derived from laser microdissected tissue samples obtained by the PALM MicroBeam system using the RNA 6000 Pico kit	5988-9128EN
Successful analysis of low RNA concentrations with the Agilent 2100 Bioanalyzer and the RNA 6000 Pico kit	5989-0712EN
Assessing genomic DNA contaminations of total RNA isolated from kidney cells obtained by laser capture microdissection using the Agilent RNA 6000 Pico assay	5989-0991EN
High sensitivity quality control of RNA samples using the RNA 6000 Pico kit	5988-8554EN

mRNA

Analysis of messenger RNA using the RNA 6000 kit	5968-7495EN
Interpreting mRNA electropherograms	5988-3001EN
Characterization of RNA quality using the RNA 6000 kit	5980-0472EN

Small RNA

Analysis of miRNA content in total RNA preparations using the Agilent 2100 Bioanalyzer	5989-7870EN
Analysis of small RNAs from Drosophila Schneider cells using the Small RNA assay on the Agilent 2100 Bioanalyzer	5989-8539EN
RNA quality control in miRNA expression analysis	5990-5557EN

Cy5 labeled samples

Analysis of Cy5-labeled cRNAs and cDNAs using the RNA 6000 kit	5980-0321EN
Optimizing cRNA fragmentation for microarray experiments using Agilent 2100 Bioanalyzer	5988-3119EN

Oncology

Gene expression profiling of esophageal cancer using laser capture microdissected samples	5989-1088EN

Technical

Performance of the Agilent Small RNA assay	5989-7002EN
New Series II RNA 6000 assays for the Agilent 2100 Bioanalyzer	5989-4571EN
Comparing performance of the Agilent 2100 Bioanalyzer to traditional RNA analysis	5980-2206EN

Protein Applications

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Optimization of protein purification using the Agilent 2100 Bioanalyzer (analysis of column capacity)	5988-4022EN
Using the Agilent 2100 Bioanalyzer for analysis of His-tag removal from recombinant proteins	5988-8144EN
Protein purification and characterization using the Agilent 1100 Series purification system and the Agilent 2100 Bioanalyzer	5988-8630EN
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Monitoring antibody charge variants using a combination of 3100 OFFGEL fractionation by isoelectric point and high sensitivity protein detection with the 2100 Bioanalyzer	5990-6521EN
High Sensitivity Detection	
Performance characteristics of the High Sensitivity Protein 250 assay for the Agilent 2100 Bioanalyzer	5989-8940EN
Immunoprecipitation and the High Sensitivity Protein 250 Assay	5990-4097EN
Additional Pico protocol for the High Sensitivity Protein 250 assay with the Agilent 2100 Bioanalyzer	5990-3703EN
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Absolute quantitation of IgG with the Protein 200 kit	5988-4021EN
Comparison of different protein quantitation methods	5988-6576EN
Analysis of bispecific antibodies using the Agilent 2100 Bioanalyzer and the Protein 200 Plus assay	5988-9651EN
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Comparison of different protein quantitation methods	5988-6576EN
Quantification strategies with the High Sensitivity Protein 250 assay	5989-8941EN
Protein Sizing and Quantitation with the Agilent Protein 80 and Protein 230 Kits on the Agilent 2100 Bioanalyzer	5990-5721EN

Food Analysis

Characterization of transgenic soybean seedlines by protein expression with the Agilent 2100 Bioanalyzer	5988-9441EN
Rapid wheat varietal identification using the Agilent 2100 Bioanalyzer and automated pattern-matching	5989-7735EN

Others

Alternative to 2D gel electrophoresis – OFFGEL electrophoresis combined with high-sensitivity on-chip protein detection	5989-8419EN
Glycoprotein sizing on the Agilent 2100 Bioanalyzer	5989-0332EN
Increased sensitivity by desalting protein samples prior to analysis on the Agilent 2100 Bioanalyzer	5989-0228EN
Using the Agilent 2100 Bioanalyzer for quality control of protein samples prior to MS-analysis	5989-0771EN

Technical

Fast analysis of proteins between 5-50 kDa using the Agilent 2100 Bioanalyzer and Protein 50 assay	5988-8322EN
Protein sizing and analysis using the Protein 200 kit	5988-0975EN
Differences and similarities between Protein 200 Assay and SDS-PAGE	5988-3160EN
Quality control for the Agilent 2100 Bioanalyzer Protein 200 Plus kits	5989-3336EN
New Series II Protein 230 assay for the Agilent 2100 Bioanalyzer	5989-4816EN
Use of lab-on-a-Chip technology for protein sizing and quantitation	5988-8604EN

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