USER GUIDE

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NuPAGE® Large Protein Analysis System

A system for electrophoresis, blotting, and staining of high molecular weight proteins

Catalog numbers LP0001, LP0002, LP0003

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life technologies™

Contents

Kit Contents and Storage

Types of Products

This manual is shipped with the following products:

Product	Quantity	Cat. no.
NuPAGE® Large Protein Blotting Kit	1 kit	LP0001
NuPAGE® Large Protein Staining Kit	1 kit	LP0002
NuPAGE® Large Protein Sensitive Staining Kit	1 kit	LP0003

NuPAGE® Large Protein Blotting Kit

The NuPAGE® Large Protein Blotting Kit contents are listed in the following table. Sufficient reagents are included to run and blot 20 mini-gels.

Store the kit contents as listed in the following table.

Component	Quantity	Storage
HiMark™ Pre-Stained High Molecular (HMW) Weight Protein Standard	250 µL	–30°C to –10°C
NuPAGE® Novex 3–8% Tris-Acetate Gel (1.0-mm, 10 wells)	20 gels	2°C to 8°C
NuPAGE® Tris-Acetate SDS Buffer Kit	1 kit (page vi)	2°C to 8°C
NuPAGE® Transfer Buffer (20X)	1 L	Room temperature, 15°C to 30°C
Nitrocellulose Membrane/Filter Sandwiches (0.45 µm)	20 sandwiches	Room temperature, 15°C to 30°C

Product Use

For research use only. Not intended for any animal or human therapeutic or diagnostic use.

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Kit Contents and Storage, Continued

NuPAGE® Large Protein Staining Kit

The NuPAGE® Large Protein Staining Kit contents are listed in the following table. Sufficient reagents are included to run and stain 20 mini-gels.

Store the kit contents as listed in the following table.

Component	Quantity	Storage
HiMark™ Pre-Stained HMW Protein Standard	250 µL	–30°C to –10°C
NuPAGE® 3–8% Tris-Acetate Gel (1.0-mm, 10 wells)	20 gels	2°C to 8°C
NuPAGE® Tris-Acetate SDS Buffer Kit	1 kit (next page)	2°C to 8°C
SimplyBlue™ SafeStain*	1 L	Room temperature, 15°C to 30°C

*Sufficient SimplyBlue™ SafeStain is included to stain 50 mini-gels.

NuPAGE® Large Protein Sensitive Staining Kit

The NuPAGE® Large Protein Sensitive Staining Kit contents are listed in the following table. Sufficient reagents are included to run and stain 20 mini-gels.

Store the kit contents as listed in the following table.

Component	Quantity	Storage
HiMark™ Unstained HMW Protein Standard	250 µL	–30°C to –10°C
NuPAGE® 3–8% Tris-Acetate Gel (1.0-mm 10 wells)	20 gels	2°C to 8°C
NuPAGE® Tris-Acetate SDS Buffer Kit	1 kit (page vi)	2°C to 8°C
SilverQuest™ Silver Staining Kit	1 kit (page vi)	Room temperature, 15°C to 30°C

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Kit Contents and Storage, Continued

NuPAGE® Tris-Acetate SDS Buffer Kit

The NuPAGE® Tris-Acetate SDS Buffer Kit contents are listed in the following table.

Store the kit at 2°C to 8°C.

Component	Amount
NuPAGE® Tris-Acetate SDS Running Buffer (20X)	500 mL
NuPAGE® LDS Sample Buffer (4X)	10 mL
NuPAGE® Antioxidant	15 mL
NuPAGE® Sample Reducing Agent (10X)	250 µL

SilverQuest™ Silver Staining Kit

The SilverQuest™ Silver Staining Kit contents are listed in the following table. Sufficient reagents are included to stain 25 mini-gels.

Store the kit at room temperature, 15°C to 30°C.

Item	Amount	Color
Sensitizer	250 mL	Orange
Stainer	25 mL	Clear
Developer	250 mL	Pink
Developer Enhancer	2 mL	Clear
Stopper	250 mL	Clear
Destainer A	60 mL	Yellow
Destainer B	60 mL	Clear

Introduction

Overview

Description

The NuPAGE® Large Protein Analysis System consists of 3 kits specifically designed for separating and analyzing high molecular weight (HMW) proteins in the molecular weight range of 30-500 kDa.

The kits include pre-cast NuPAGE® Novex Tris-Acetate Gels and buffers for high-resolution electrophoresis of HMW proteins in the neutral pH range (page 3). The pre-cast gels are designed for use with the XCell *SureLock*® Mini-Cell.

High Molecular Weight (HMW) Protein Analysis

The HMW proteins are involved in a large variety of important pathways such as cell signaling, nuclear protein transport, DNA repair, and in disease states such as cancer.

The NuPAGE® Large Protein Analysis System allows you to rapidly and efficiently separate high molecular weight proteins on polyacrylamide gels and provides sensitive stains for detection. The kits also include a protein standard specifically designed for analyzing high molecular weight proteins and allow you to easily determine protein molecular weight in the range of 30–500 kDa.

System Overview

High molecular weight protein samples are prepared using the NuPAGE® Sample Buffer and electrophoresed on NuPAGE® Novex Tris-Acetate Gels with the NuPAGE® Tris-Acetate SDS Running Buffer using the XCell *SureLock*® Mini-Cell.

Based on the kit that you have purchased, you can stain or blot the proteins after electrophoresis. The proteins can be stained using a Coomassie based SimplyBlue™ SafeStain (NuPAGE® Large Protein Staining Kit) or a sensitive SilverQuest™ Silver stain, (NuPAGE® Large Protein Sensitive Silver Staining Kit). The proteins can also be subjected to western transfer onto the nitrocellulose membrane with the NuPAGE® Transfer Buffer (NuPAGE® Large Protein Blotting Kit) using the XCell II™ Blot Module.

Continued on next page

Overview, Continued

NuPAGE® Large Protein Blotting Kit

The NuPAGE® Large Protein Blotting Kit is ideal for Western blotting of high molecular weight proteins. The kit includes pre-cast gels, pre-made buffers, and pre-assembled nitrocellulose membrane/filter paper sandwiches to ensure consistent results and eliminates the need to prepare reagents. A pre-stained protein standard (HiMark™) specifically designed for analyzing high molecular weight proteins is also included to allow you to easily monitor the protein transfer.

This kit is designed for use with the XCell II™ Blot Module.

NuPAGE® Large Protein Staining Kit

The NuPAGE® Large Protein Staining Kit provides a fast and sensitive method of protein detection using the SimplyBlue™ SafeStain. The kit includes pre-cast gels, pre-made buffers, SimplyBlue™ SafeStain, and a pre-stained HMW protein standard (HiMark™). The stain can detect protein bands at nanogram level, is safe, non-hazardous, and easy to dispose.

Proteins stained using SimplyBlue™ SafeStain are compatible with mass spectrometry analysis.

NuPAGE® Large Protein Sensitive Staining Kit

The NuPAGE® Large Protein Sensitive Staining Kit provides a rapid and easy method to silver stain proteins and allows you to detect protein bands at nanogram level. The kit includes pre-cast gels, pre-made buffers, SilverQuest™ Silver Staining Kit, and an unstained HMW protein standard (HiMark™). The SilverQuest™ Silver Staining Kit is specifically designed to provide sensitive silver staining compatible with mass spectrometry analysis.

Purpose of the Manual

This manual provides the following information:

- An overview of the NuPAGE® Large Protein Analysis System
- Instructions for electrophoresis of proteins
- Protocols for staining using the SimplyBlue™ Safestain and SilverQuest™ Silver Staining Kit
- Western blotting protocol using the XCell II™ Blot Module

For more details, refer to the NuPAGE® Technical Guide available from **www.lifetechnologies.com** or contact Technical Support (page 26).

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NuPAGE® Electrophoresis System

Introduction

The NuPAGE® Large Protein Analysis System Kits are supplied with high-performance NuPAGE® Novex 3–8% Tris-Acetate Gels and NuPAGE® Tris-Acetate SDS Buffer Kit for optimal resolution of high molecular weight proteins.

NuPAGE® Tris-Acetate Gels

The NuPAGE® Novex 3–8% Tris-Acetate Pre-Cast Gel is a 1.0 mm thick, 10 × 10 cm mini-gel used for electrophoresis of high molecular weight proteins. The gels resolve proteins in the molecular weight range of 30–500 kDa.

The NuPAGE® Novex Tris-Acetate Pre-Cast Gels are used with the NuPAGE® Tris-Acetate SDS Buffer System (see the following section) to produce a discontinuous SDS-PAGE system operating at neutral pH. The neutral pH environment during electrophoresis results in maximum stability of both proteins and gel matrix, providing better band resolution than other gel systems.

For more details, refer to the NuPAGE® Technical Guide available at **www.lifetechnologies.com**, or contact Technical Support (page 26).

NuPAGE® Tris-Acetate Buffer System

The NuPAGE® Tris-Acetate discontinuous buffer system involves three ions:

- Acetate ($^-$) is supplied by the gel buffer and serves as a leading ion due to its high affinity to the anode as compared to other anions in the system. The gel buffer ions are Tris ($^+$) and Acetate ($^-$), pH 7.0.
- Tricine ($^-$) serves as the trailing ion from the running buffer. The running buffer ions are Tris$^+$, Tricine$^-$, and dodecylsulfate ($^-$), pH 8.3.
- Tris ($^+$) is the common ion present in the gel buffer and running buffer. The Tris-Acetate system also operates at a significantly lower operating pH of 8.1 during electrophoresis.

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NuPAGE® Electrophoresis System,
Continued

Advantages

The operating neutral pH of NuPAGE® Gels and buffers provide the following advantages over the Laemmli system:

- Longer shelf life of 8 months due to improved gel stability
- Improved protein stability during electrophoresis at neutral pH resulting in sharper band resolution and accurate results
- Complete reduction of disulfides under mild heating conditions (70°C for 10 minutes) and absence of cleavage of asp-pro bonds using the NuPAGE® LDS Sample buffer (pH > 7.0 at 70°C)
- Reduced state of the proteins maintained during electrophoresis and blotting of the proteins by the NuPAGE® Antioxidant

NuPAGE® LDS Sample Buffer

Use the NuPAGE® LDS Sample Buffer (4X) to prepare samples for denaturing gel electrophoresis with the NuPAGE® Tris-Acetate Gels.

Since the pH of the NuPAGE® LDS Sample Buffer is 8.4, sample reduction at slightly alkaline pH allows for maximal activity of the reducing agent.

NuPAGE® Reducing Agent

The NuPAGE® Reducing Agent contains 500 mM dithiothreitol (DTT) at a 10X concentration and is available in a ready-to-use, stabilized liquid form (page 25). Use the NuPAGE® Reducing Agent to prepare samples for reducing gel electrophoresis.

We recommend adding the reducing agent to the sample within an hour of loading the gel.

Avoid storing reduced samples for long periods even if they are frozen. This will result in the reoxidation of samples during storage and produce inconsistent results.

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NuPAGE® Electrophoresis System,
Continued

NuPAGE® Antioxidant

The reducing agents, DTT and β-mercaptoethanol, do not co-migrate through the gel with the sample in a neutral pH environment of the NuPAGE® Gels. Instead, the reducing agent tends to remain at the top of the gel and not migrate fully throughout the gel resulting in the reoxidization of some proteins producing slightly diffuse bands.

The NuPAGE® Antioxidant (a proprietary reagent) is added to the running buffer in the upper (cathode) buffer chamber only when performing electrophoresis under reducing conditions. The NuPAGE® Antioxidant migrates with the proteins during electrophoresis preventing the proteins from reoxidizing and maintaining the proteins in a reduced state. The NuPAGE® Antioxidant also protects sensitive amino acids such as methionine and tryptophan from oxidizing.

Important

Do not use the NuPAGE® Antioxidant as a sample reducing agent. The antioxidant is not efficient in reducing the disulfide bonds. This will result in partially reduced bands with substantial background smearing in the lane.

The antioxidant maintains the sample proteins that have been previously reduced with a reducing agent in a reduced state and prevents the proteins from reoxidizing during electrophoresis.

Methods

Perform SDS-PAGE

Introduction

Instructions to perform SDS-PAGE under denaturing conditions using the XCell *SureLock*® Mini-Cell are described in this section.

HiMark™ Protein Standard

The HiMark™ High Molecular Weight (HMW) Protein Standard allows you to estimate the molecular weight of high molecular weight proteins on NuPAGE® Novex Tris-Acetate Gels with Tris-Acetate SDS buffer system. The standard is supplied in a ready-to-use format and there is no need to heat or reduce the standard prior to use.

Use the appropriate HiMark™ HMW Protein Standard based on your application:

- HiMark™ Pre-Stained HMW Protein Standard is included with the Large Protein Blotting and Staining Kits and allows you to easily visualize protein molecular weight ranges during electrophoresis and evaluate western transfer efficiency.
- HiMark™ Unstained HMW Protein Standard is supplied with Large Protein Sensitive Staining Kit and allows you to accurately estimate molecular weight. Use Coomassie or silver staining after electrophoresis or Ponceau S, Coomassie, SYPRO® Ruby Blot Stain, or other membrane stains after western transfer to visualize the unstained standard.

  A molecular weight calculator is available from **www.lifetechnologies.com** to allow you to easily and accurately calculate the molecular weight of your proteins on NuPAGE® Novex Tris-Acetate Gels and to extrapolate the molecular weight of proteins beyond the standard curve.

Continued on next page

Perform SDS-PAGE, Continued

Gels are individually packaged in clear pouches with 10 mL of Packaging Buffer. The Packaging Buffer contains low levels of residual acrylamide monomer and 0.02% sodium azide. Wear gloves at all time when handling gels.

Warning: This product contains a chemical (acrylamide) known to the state of California to cause cancer. To obtain a SDS, see page 26.

Note

Sufficient reagents are included in the NuPAGE® Tris-Acetate Buffer Kit to run 20 mini-gels when 2 mini-gels are used per electrophoresis run and 10 µL sample/well is prepared. You may need additional reagents from the buffer kit if you are using 1 mini-gel per run or preparing 25 µL sample/well. Ordering information is on page 25.

Required Materials

Materials supplied by the user

- Protein sample
- Deionized water
- XCell *SureLock*® Mini-Cell (page 25)

*Components included in the kit (*see the preceding note)

- NuPAGE® 3–8% Tris-Acetate Gel (1.0-mm, 10 wells)
- NuPAGE® Tris-Acetate SDS Buffer Kit
- Appropriate HiMark™ HMW Protein Standard

Continued on the next page

Perform SDS-PAGE, Continued

Prepare 1X Running Buffer

You will need 1000 mL 1X NuPAGE® Tris-Acetate SDS Running Buffer for electrophoresis with the XCell *SureLock*® Mini-Cell.

1. Prepare 1000 mL 1X NuPAGE® SDS Running Buffer using NuPAGE® SDS Running Buffer (20X):

NuPAGE® Tris-Acetate SDS Buffer (20X)	50 mL
Deionized Water	950 mL
Total Volume	1000 mL

2. Mix thoroughly and set aside 800 mL of the 1X NuPAGE® SDS Running Buffer for use in the Lower (Outer) Buffer Chamber of the XCell *SureLock*® Mini-Cell.
3. Immediately prior to electrophoresis, add 500 µL of NuPAGE® Antioxidant to 200 mL of 1X NuPAGE® SDS Running Buffer from step 1 of this procedure for use in the Upper (Inner) Buffer Chamber of the XCell *SureLock*® Mini-Cell. Mix thoroughly.

Prepare Samples

The recommended protein load for a 10-well gel is 0.5 µg/band for Coomassie staining and 1 ng/band for silver staining. For western detection, load the amount of protein according to the sensitivity of your detection method.

Prepare your samples in a total volume of 10 µL:

Note: If you need to prepare samples in a volume of 5-25 µL, adjust the volume accordingly.

1. To a sterile microcentrifuge tube, add the following:

Protein Sample	*x* µL
NuPAGE® LDS Sample Buffer (4X)	2.5 µL
NuPAGE® Reducing Agent (10X)	1 µL
Deionized Water	to 10 µL

2. Heat samples at 70°C for 10 minutes. Immediately load the samples on the gel, page 9.

Continued on next page

Perform SDS-PAGE, Continued

Load Samples

1. Assemble the XCell *SureLock*® Mini-Cell as described in the manual supplied with the unit. The manual is available from **www.lifetechnologies.com** or by contacting Technical Support (page 26).
2. Fill the Upper Buffer Chamber (inner) of the unit with 200 mL 1X running buffer with antioxidant (see page 8 for instructions to prepare running buffer). The buffer level must exceed the level of the wells.
3. Load 10–25 µL sample at the intended protein concentration onto each well of the gel.
4. Load HiMark™ Protein Standard as follows:
   - For Coomassie staining and Western blotting, use **10 µL** HiMark™ **Pre-Stained** HMW Protein Standard
   - For silver staining, use **5 µL of 1:10 diluted** HiMark™ **Unstained** HMW Protein Standard
5. Fill the Lower (outer) Buffer Chamber with 600 mL 1X running buffer (see page 8).
6. Perform electrophoresis at constant voltage of 150 V for 1 hour at room temperature.

   The expected start current is 40–55 mA/gel and the expected end current is 25–40 mA/gel.
7. After electrophoresis is complete, disassemble the XCell *SureLock*® Mini-Cell and remove the gel from the cassette as described in the XCell *SureLock*® Mini-Cell manual.
8. Proceed to blotting (page 10) or staining (page 15).

Western Blot

Introduction

The NuPAGE® Large Protein Blotting Kit includes reagents to perform electrophoresis and blotting of high molecular weight proteins.

Instructions for western blotting using the XCell II™ Blot Module and nitrocellulose membranes are described in this section.

For more details, refer to the XCell II™ Blot Module manual available from **www.lifetechnologies.com**, or contact Technical Support (page 26).

Nitrocellulose Membrane

The Nitrocellulose membrane/filter paper sandwich (0.45 µm) included with the NuPAGE® Large Protein Blotting Kit contains 100% pure nitrocellulose to provide high-quality transfer with high-sensitivity and low background. The membrane is supplied in a pre-cut, pre-assembled membrane/filter paper sandwich for easy assembly and is compatible with detection methods such as staining, immunodetection, fluorescence, or radiolabeling.

The 0.45 µm Nitrocellulose Membrane has a binding capacity of 80 µg/cm^2 of proteins and is ideal for the transfer of most large proteins (>20 kDa).

NuPAGE® Transfer Buffer

The NuPAGE® Transfer Buffer (included with the kit) is recommended for western transfer of proteins from NuPAGE® Tris-Acetate Gels. The transfer buffer maintains the neutral pH environment established during gel electrophoresis, protects against modification of the amino acid side chains and is compatible with N-terminal protein sequencing using Edman degradation.

Note

The NuPAGE® Large Protein Blotting Kit is supplied with 0.45 µm nitrocellulose membranes. However, you can also use PVDF membranes (0.45 µm) for Western blotting using the protocol described in this section. PVDF membranes are available separately (page 25).

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Western Blot, Continued

Required Materials

Materials supplied by the user

- Methanol
- XCell II™ Blot Module (page 25)
- Deionized water
- Optional: PVDF membranes (page 25)

Components included with the kit

- Nitrocellulose membrane/filter paper sandwich
- NuPAGE® Transfer Buffer
- NuPAGE® Antioxidant

Wear gloves at all times during the entire blotting procedure to prevent contaminating gels and membranes, and to avoid exposing your skin to irritants commonly used in electrophoresis and blotting procedures.

Do not touch the membrane or gel with bare hands. This may contaminate the gel or membrane and interfere with further analysis.

Prepare 1X Transfer Buffer

Prepare 1000 mL of 1X NuPAGE® Transfer Buffer with 10% methanol* using the NuPAGE® Transfer Buffer (20X) supplied with the kit:

NuPAGE® Transfer Buffer (20X)	50 mL
NuPAGE® Antioxidant	1 mL
Methanol	100 mL
Deionized Water	849 mL
Total Volume	1000 mL

*Prepare NuPAGE® Transfer Buffer with 20% methanol, if you are using HiMark™ Pre-stained Protein Standard with PVDF membranes or performing transfer of 2 gels in the blot module to obtain optimal transfer.

Continued on next page

Western Blot, Continued

Prepare Blotting Pads, Membrane, and Filter Paper

- **Blotting Pads:** Use ~ 700 mL of transfer buffer to soak the blotting pads until saturated. Remove air bubbles by squeezing the blotting pads while they are submerged in buffer. Removing air bubbles is essential because they can block the transfer of biomolecules.
- **Nitrocellulose membrane**: Place the nitrocellulose membrane (supplied with the kit) directly in a tray containing the transfer buffer for several minutes.

  Note: If you are using PVDF membranes for blotting, pre-wet the PVDF membrane for 30 seconds in methanol, ethanol, or isopropanol. Briefly rinse in deionized water and then place the membrane in a tray containing transfer buffer for several minutes.
- **Filter paper**: Soak briefly in transfer buffer immediately prior to use.

Transfer 1 Gel

For transferring 1 gel using the XCell II™ Blot Module:

1. After electrophoresis (page 9), remove gel from the cassette. Use the gel immediately without soaking the gel in transfer buffer.
2. Place a piece of pre-soaked filter paper on top of your gel, and remove any trapped air bubbles. Turn the gel/filter paper over so the gel is facing up.
3. Place pre-soaked nitrocellulose membrane on the gel. Roll a glass pipette over the membrane surface to remove any air bubbles.
4. Place the other pre-soaked filter paper on top of the transfer membrane. Remove any trapped air bubbles.
5. Place 2 soaked blotting pads onto the cathode (-) core of the blot module.

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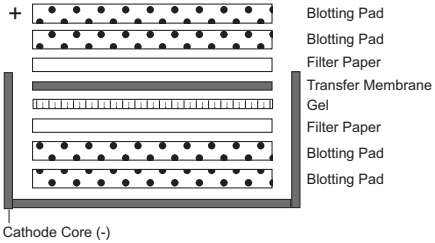
Western Blot, Continued

Transfer 1 Gel, Continued

6. Carefully pick up the gel/membrane assembly with your gloved hand and place on the pad in the same sequence, such that the gel is closest to the cathode plate:



7. Add enough pre-soaked blotting pads to rise 0.5 cm over the rim of the cathode core. Place the anode (+) core on top of the pads.
8. Hold the blot module together firmly and slide it into the guide rails on the lower buffer chamber.
9. Insert the Gel Tension Wedge into the Lower Buffer Chamber and lock the Wedge into position.
10. Fill the blot module with 1X NuPAGE® Transfer Buffer until the gel/membrane assembly is covered.
11. Fill the Outer Buffer Chamber with 650 mL deionized water.
12. Attach the lid, connect the leads to the power supply, and perform Western transfer using conditions listed below.

Note: To perform Western transfer of 2 gels, refer to the XCell II™ Blot Module manual. This manual is available by contacting Technical Support (page 26) or from **www.lifetechnologies.com**.

Transfer Conditions

Voltage:	30 V constant
Time:	1 hour
Expected Current:	220 mA/gel (start of run) 180 mA/gel (end of run)
Overnight blotting Voltage:	10–15 V constant
Run Time:	Overnight

For overnight blotting, perform transfer in the cold room to prevent overheating.

Continued on next page

Western Blot, Continued

Transfer Efficiency

You can monitor the transfer of HiMark™ Pre-Stained HMW Protein Standard bands to determine the transfer efficiency. If most of the standard bands have transferred to the membrane, then transfer efficiency is good.

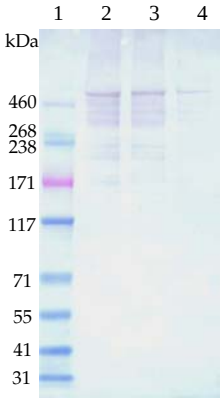
It is important to note that high molecular weight standards do not always transfer completely and is not indicative of an incomplete transfer.

If none of the standard bands or only a few of standard bands have transferred, or the transfer of your protein of interest is not complete, you may have to optimize the transfer conditions as described in the XCell II™ Blot Module manual.

Expected Results

See the following image for an example of a Western blot of HiMark™ Pre-Stained HMW Protein Standard and an ~ 500 kDa human protein run on a NuPAGE® Novex 3–8% Tris-Acetate Gel and transferred onto a nitrocellulose membrane using the protocol described in this manual. The proteins were detected using the WesternBreeze® Chromogenic Kit with 1:500 dilution of anti-human protein antibody. The blot was developed for ~5 minutes with the chromogenic substrate.

Efficient transfer of the standard bands and high molecular weight human protein is observed.



Lane 1: 10 µL HiMark™ Pre-stained Standard

Lane 2: 500 ng human protein

Lane 3: 300 ng human protein

Lane 4:100 ng human protein

Use SimplyBlue™ SafeStain

Introduction

The NuPAGE® Large Protein Staining Kit includes the reagents to perform electrophoresis and staining with SimplyBlue™ SafeStain.

Instructions for staining gels with SimplyBlue™ SafeStain are included in this section. For more details, refer to the SimplyBlue™ SafeStain manual available from **www.lifetechnologies.com** or contact Technical Support (page.26).

SimplyBlue™ SafeStain

SimplyBlue™ SafeStain included with the NuPAGE® Large Protein Staining Kit is a ready-to-use, proprietary Coomassie G-250 stain that is specially formulated for fast, sensitive detection and safe, non-hazardous disposal. Proteins stained using the SimplyBlue™ SafeStain are compatible with mass spectrometry (MS) analysis.

Required Materials

Materials supplied by the user:

- Staining containers
- 20% NaCl (w/v) in deionized water
- Shaker
- Deionized water
- **Optional:** Microwave oven

Components included with the kit:

- SimplyBlue™ SafeStain

Continued on next page

Use SimplyBlue™ SafeStain, Continued

Basic Protocol

After electrophoresis, follow the instructions below. Be sure the mini-gel moves freely in water or stain to facilitate diffusion during all steps.

An alcohol/acetic acid fixing step prior to staining with SimplyBlue™ SafeStain is **NOT** required or recommended.

1. **Rinse** the mini-gel 3 times for 5 minutes with 100 mL deionized water to remove SDS and buffer salts, which interfere with binding of the dye to the protein. Discard each rinse.
2. **Stain** the mini-gel with enough SimplyBlue™ SafeStain (~20 mL) to cover the mini-gel. Stain for 1 hour at room temperature with gentle shaking. Bands will begin to develop within minutes. After incubation, discard the stain. Do not re-use the stain.

   Note: The gel can be stained for up to 3 hours, but after 3 hours, sensitivity will decrease. If you need to leave the gel overnight in the stain, add 2 mL 20% NaCl (w/v) in water for every 20 mL of stain. This procedure will not affect sensitivity.
3. **Wash** the mini-gel with 100 mL water for 1-3 hours. The gel can be left in the water for several days without loss of sensitivity.
   - **To obtain the highest sensitivity:** After washing the gel for 1 hour, add 20 mL 20% NaCl solution to the water and continue to wash for 2 hours up to overnight. **(Detection limit: 7 ng BSA.)**
   - **To obtain the clearest background for photography:** Perform a second 1 hour wash with 100 mL water.

   Note: Once in the second wash, sensitivity will decrease if the gel is allowed to stay in the water more than 1 day. If you need to store the gel in water for a few days, add 20 mL 20% NaCl to the solution.

Continued on next page

Use SimplyBlue™ SafeStain, Continued

Microwave Protocol

The microwave protocol is fast, takes just 12 minutes, and can yield results with sensitivity as low as 5 ng with an additional incubation with a salt solution.

Note: Use caution while using the stain in a microwave oven. Do not overheat the staining solutions.

1. After electrophoresis, place the gel in 100 mL deionized water in a loosely covered container and microwave on High (950 to 1100 watts) for 1 minute until the solution almost boils.
2. Shake the gel on an orbital shaker for 1 minute. Discard the water.
3. Repeat Steps 1–2 two more times.
4. After the last wash, add 20 mL SimplyBlue™ SafeStain and microwave on High for 45 seconds to 1 minute until the solution almost boils.
5. Shake the gel on an orbital shaker for 5 minutes. **(Detection limit: 20 ng BSA.)**
6. Wash the gel in 100 mL of ultrapure water for 10 minutes on a shaker. **(Detection limit: 10 ng BSA.)**
7. Add 20 mL of 20% NaCl for at least 5 minutes. **(Detection limit: 5 ng BSA.)** The gel can be stored for several weeks in the salt solution.

Disposal Information

In most cases, the SimplyBlue™ SafeStain can be diluted 1:200 with water and flushed down the drain. Diluting the stain raises the pH to between 5 and 7, which complies with most federal and state regulations. For disposal requirements in your area, consult your safety officer.

Dry the SimplyBlue™ SafeStain Gel

The stained gel can be dried for storage or analysis by vacuum-drying or air-drying. We recommend using the DryEase® Mini-Gel Drying System (page 25) to air-dry the gel.

When using the DryEase® Mini-Gel Drying System, be sure to restrict the incubation time of your SimplyBlue™ SafeStain stained gel in the Gel-Dry™ Drying Solution to 5 minutes because longer exposure can result in destaining of bands.

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Use SimplyBlue™ SafeStain, Continued

Expected Results

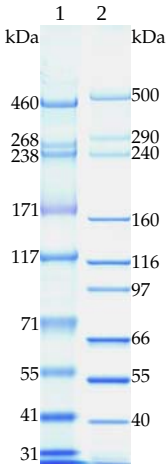
An example of protein standards analyzed on a NuPAGE® Novex 3–8% Tris-Acetate Gel and stained with SimplyBlue™ SafeStain Kit using the protocol described in this manual is shown below.

The samples on the gel are:

Lane 1: 10 µL of HiMark™ Pre-Stained Standard

Lane 2: 5 µL of HiMark™ Unstained Standard

Note: The red, 171 kDa band from HiMark™ Pre-stained Standard turns blue upon staining with SimplyBlue™ SafeStain.



Use the SilverQuest™ Silver Staining Kit

Introduction

The NuPAGE® Large Protein Sensitive Staining Kit includes the reagents to perform electrophoresis and staining with SilverQuest™ Silver Staining Kit.

Instructions for staining the gels with SilverQuest™ Silver Staining Kit are included in this section. For more details, refer to the SilverQuest™ Silver Staining Kit manual available from **www.lifetechnologies.com** or contact Technical Support (page.26).

SilverQuest™ Staining Kit

The SilverQuest™ Silver Staining Kit (included with the NuPAGE® Large Protein Sensitive Staining Kit) is a rapid and easy method for silver staining of proteins. Silver staining allows you to detect most proteins at sub-nanogram levels. The SilverQuest™ Kit is specifically designed to provide sensitive silver staining of large proteins and is compatible with mass spectrometry analysis. The kit also includes a specially formulated sensitizer to provide improved sensitivity and light background when compared to other silver staining methods.

Note

The SilverXpress® Silver Staining Kit available separately or other similar silver staining kits do not properly stain large proteins on NuPAGE® Tris-Acetate Gels.

To obtain the best results for silver staining of large proteins on NuPAGE® Tris-Acetate Gels, use the SilverQuest™ Silver Staining Kit supplied with the NuPAGE® Large Protein Sensitive Staining Kit.

- Always use ultrapure water of >18 megohm/cm resistance, clean glass containers, and Teflon-coated stir bars for preparing and handling all solutions
- Make sure the mini-gel moves freely and is fully immersed in solutions during shaking in each step
- Use freshly made solutions
- Avoid touching the gel with bare hands or metal objects
- Be sure to keep to the volumes and incubation times of steps as described in the protocol

Continued on next page

Use the SilverQuest™ Silver Staining Kit, Continued

Required Materials

Materials supplied by the user

- Ultrapure water (>18 megohm/cm resistance recommended, see page 19)
- Staining tray (a polypropylene tray is recommended)
- Rotary shaker
- Clean glass bottles for reagent preparation
- 30% ethanol (made with ultrapure water)
- 100% ethanol
- Fixative (40% ethanol, 10% acetic acid, made with ultrapure water)

Components included

- SilverQuest™ Silver Staining Kit

Prepare Staining Solutions

Prepare the following solutions for use with the basic or microwave protocols.

Use the reagents provided in the kit to prepare the following solutions for staining:

- Sensitizing solution

Ethanol	30 mL
Sensitizer	10 mL
Ultrapure water	to 100 mL

- Staining solution

Stainer	1 mL
Ultrapure water	to 100 mL

- Developing solution

Developer	10 mL
Developer enhancer	1 drop
Ultrapure water	to 100 mL

Note: You may prepare all solutions immediately before starting the staining protocol or prepare them as you proceed to the next step.

Continued on next page

Use the SilverQuest™ Silver Staining Kit, Continued

Basic Protocol

The staining time using the Basic Protocol is **90 minutes**.

Perform all incubations on a rotary shaker rotating at a speed of 1 revolution/sec at room temperature. **Be sure to use 100 mL of each solution per gel**.

1. After electrophoresis, remove the gel from the cassette and place the gel in a clean staining tray. Rinse the gel briefly with ultrapure water.
2. Fix the gel in 100 mL fixative for 20 minutes with shaking.

   Note: The gel can be stored in the fixative overnight if there is not enough time to complete the staining protocol. Longer fixing times may improve the sensitivity and background staining in some cases.
3. Decant the fixative solution and wash the gel in 30% ethanol for 10 minutes.
4. Decant the ethanol and add 100 mL Sensitizing solution. Incubate for 10 minutes.
5. Decant the Sensitizing solution and wash the gel in 100 mL 30% ethanol for 10 minutes.
6. Wash the gel in 100 mL ultrapure water for 10 minutes.
7. Incubate the gel in 100 mL Staining solution for 15 minutes.
8. After staining is complete, decant the Staining solution and wash the gel with 100 mL ultrapure water for 20-60 seconds. **Note**: Washing the gel for more than 1 minute will remove silver ions from the gel resulting in decreased sensitivity.
9. Incubate the gel in 100 mL Developing solution for 4-8 minutes until bands start to appear and the desired band intensity is reached.
10. When the desired staining intensity is achieved, immediately add 10 mL Stopper directly to the gel still immersed in Developing solution. Gently agitate the gel for 10 minutes. The color changes from pink to colorless indicating the end of development.
11. Decant the Stopper solution and wash the gel with 100 mL ultrapure water for 10 minutes.
12. Proceed to destaining or drying the gel (page 23).

Continued on next page

Use the SilverQuest™ Silver Staining Kit, Continued

Microwave Protocol

The staining time using the microwave protocol is **~1 h**. **Be sure to use 100 mL of each solution per gel.**

Note: Use caution while using the stain in a microwave oven. Do not overheat the staining solutions.

1. After electrophoresis, remove the gel from the cassette and place the gel in a clean staining tray. Rinse the gel briefly with ultrapure water.
2. Place the gel in 100 mL fixative and microwave at high power (700 watts) for 30 seconds. Remove the gel from the microwave and incubate for 5 minutes at room temperature. Decant the fixative.
3. Wash the gel with 100 mL 30% ethanol in a microwave at high power for 30 seconds. Remove the gel from the microwave and incubate for 5 minutes at room temperature. Decant the ethanol.
4. Add 100 mL Sensitizing solution to the washed gel. Microwave at high power for 30 seconds. Remove gel from the microwave and incubate for 2 minutes at room temperature. Decant the Sensitizing solution.
5. Wash the gel twice in 100 mL ultrapure water. Microwave at high power for 30 seconds. At each wash step, remove the gel from microwave and incubate for 2 minutes at room temperature.
6. Place the gel in 100 mL Staining solution. Microwave at high power for 30 seconds. Remove gel from microwave and incubate for 5 minutes at room temperature.
7. Decant the Staining solution and wash the gel with 100 mL ultrapure water for 20–60 seconds. Do not wash the gel for more than 1 minute (see page 21).
8. Place the gel in 100 mL Developing solution and incubate for 5 minutes at room temperature. **Do not microwave**.
9. Once the desired band intensity is achieved, immediately add 10 mL Stopper directly to the gel still immersed in Developing solution and agitate the gel for 10 minutes. The color changes from pink to clear.
10. Wash the gel with 100 mL ultrapure water for 10 minutes.
11. Proceed to destaining or drying the gel (page 23).

Continued on the next page

Use the SilverQuest™ Silver Staining Kit, Continued

Destaining Protocol

1. After silver staining the gel, wash the gel thoroughly with ultrapure water.
2. Carefully excise the band of interest using a clean scalpel and place the gel piece into a 1.5-mL sterile microcentrifuge tube.
3. Add 50 µL of Destainer A and 50 µL of Destainer B (supplied with the kit) to each microcentrifuge tube.

   Note: If you need to destain a large number of gel bands, then prepare the required amount of the destaining solution by mixing Destainer A and B, and use immediately. Destainer solutions A and B cannot be stored for long periods after they are mixed.
4. Mix the contents of the tube thoroughly and incubate for 15 minutes at room temperature. The gel pieces will slowly settle to the bottom.
5. Remove the supernatant using a clean pipette tip.
6. Add 200 µL ultrapure water to the tube and mix. Incubate for 10 minutes at room temperature.
7. Repeat Steps 6–7 at least 2 times. Use the destained gel piece for trypsin digestion and mass spectrometry.

Dry the SilverQuest™ Silver Stained Gel

You may dry the silver stained gel by vacuum drying or by air-drying. We recommend using the DryEase® Mini-Gel Drying System (page 25) to air-dry the gel. Prior to drying the gel, wash the gel in ultrapure water for 10 minutes with gentle agitation to remove the Stopper.

If you are using vacuum drying, follow the manufacturer's instructions.

Continued on next page

Use the SilverQuest™ Silver Staining Kit,
Continued

Expected Results

An example of proteins analyzed on a NuPAGE® Novex 3–8% Tris-Acetate Gel and stained with SilverQuest™ Silver Staining Kit using the protocol described in this manual is shown below.

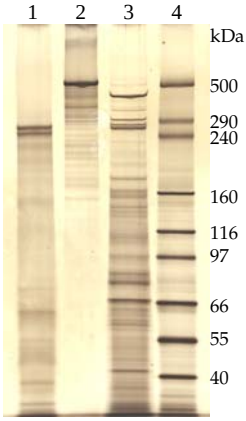
The samples on the gel are:

Lane 1: 188 ng Fibronectin

Lane 2: 40 ng of ~500 kDa human protein

Lane 3: 8 µL of human kinase

Lane 4: 5 µL of 1:10 diluted HiMark™ Unstained HMW Protein Standard



Appendix

Accessory Products

Additional Products

Ordering information for electrophoresis products available separately is provided below. For detailed information, visit **www.lifetechnologies.com** or call Technical Support (page 26).

Product	Quantity	Cat. no.
XCell *SureLock*® Mini-Cell	1 unit	EI0001
XCell II™ Blot Module	1 unit	EI9051
NuPAGE® 3–8% Tris-Acetate Gels 1.0 mm/10 well	10 gel/box	EA0375BOX
NuPAGE® 3–8% Tris-Acetate Gels 1.0 mm/12 well	10 gel/box	EA03752BOX
NuPAGE® Tris-Acetate SDS Buffer Kit	1 kit	LA0050
NuPAGE® Tris-Acetate SDS Running Buffer (20X)	500 mL	LA0041
NuPAGE® Sample Reducing Agent (10X)	250 µL	NP0004
NuPAGE® Antioxidant	15 mL	NP0005
NuPAGE® LDS Sample Buffer (4X)	10 mL	NP0007
NuPAGE® Transfer Buffer (20X)	1 L	NP0006-1
Nitrocellulose 0.45 µm Membrane/Filter Paper Sandwich	20 Sandwiches	LC2001
Invitrolon™ PVDF (0.45 µm) Membrane/Filter Paper Sandwich	20 Sandwiches	LC2005
WesternBreeze® Chromogenic Kit, Anti-Mouse	1 kit	WB7103
WesternBreeze® Chromogenic Kit Anti-Rabbit	1 kit	WB7105
WesternBreeze® Chemiluminescent Kit, Anti-Mouse	1 kit	WB7104
WesternBreeze® Chemiluminescent Kit, Anti-Rabbit	1 kit	WB7106
DryEase® Mini-Gel Drying System	1 kit	NI2387
SimplyBlue™ SafeStain	1 L	LC6060
SilverQuest™ Silver Staining Kit	1 kit	LC6070
HiMark™ Pre-Stained HMW Protein Standard	250 µL	LC5699
HiMark™ Unstained HMW Protein Standard	250 µL	LC5688

Technical Support

Obtaining support

For the latest services and support information for all locations, go to **www.lifetechnologies.com/support.**

At the website, you can:

- Access worldwide telephone and fax numbers to contact Technical Support and Sales facilities
- Search through frequently asked questions (FAQs)
- Submit a question directly to Technical Support (**techsupport@lifetech.com**)
- Search for user documents, Safety Data Sheets (SDSs), vector maps and sequences, application notes, formulations, handbooks, certificates of analysis, citations, and other product support documents
- Obtain information about customer training
- Download software updates and patches

Safety Data Sheets (SDS)

Safety Data Sheets (SDSs) are available at **www.lifetechnologies.com/support.**

Certificate of Analysis

The Certificate of Analysis provides detailed quality control and product qualification information for each product. Certificates of Analysis are available on our website. Go to **www.lifetechnologies.com/support** and search for the Certificate of Analysis by product lot number, which is printed on the box.

Limited Product Warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale found on Life Technologies' website at **www.lifetechnologies.com/termsandconditions**. If you have any questions, please contact Life Technologies at **www.lifetechnologies.com/support.**

Purchaser Notification

Limited Use Label License: Research Use Only

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