



PRODUCT INFORMATION

Thermo Scientific

GeneJET Endo-free Plasmid Maxiprep Kit

#K0861

Lot ____

Expiry Date _



CERTIFICATE OF ANALYSIS

Thermo Scientific™ GeneJET™ Endo-free Plasmid Maxiprep Kit is qualified by isolating high copy number plasmid DNA from 350 mL of overnight *E. coli* culture ($OD_{600} = 2$) grown in LB medium following the protocol outlined in the manual. The kit passes QC requirements if the purified plasmid DNA has an A_{260}/A_{280} ratio between 1.8 and 1.9 and a dominant band of supercoiled plasmid DNA is observed after agarose gel electrophoresis. The functional quality of the plasmid DNA is evaluated by digestion with restriction endonucleases.

Quality authorized by:



Jurgita Zilinskiene

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COMPONENTS OF THE KIT

GeneJET Endo-free Plasmid Maxiprep Kit	#K0861 10 preps
Resuspension Solution for GeneJET Endo-free Plasmid Maxiprep Kit	84 mL
Lysis Solution for GeneJET Endo-free Plasmid Maxiprep Kit	84 mL
Neutralization Solution for GeneJET Endo-free Plasmid Maxiprep Kit	84 mL
Endotoxin Binding Reagent for GeneJET Endo-free Plasmid Maxiprep Kit	12 mL
Wash Solution I (concentrated) for GeneJET Endo-free Plasmid Maxiprep Kit	75 mL
Wash Solution II (concentrated) for GeneJET Endo-free Plasmid Maxiprep Kit	75 mL
RNase A Solution	3 × 1.2 mL
Elution Buffer for GeneJET Endo-free Plasmid Maxiprep Kit	18 mL
GeneJET Endo-free Maxi Purification Columns & Collection tubes, blue-capped (50 mL)	10
GeneJET Endo-free Maxi Filtration Columns & Collection tubes, white-capped (50 mL)	10

STORAGE AND STABILITY

Store Endotoxin Binding Reagent at 4°C. Other components of the kit should be stored at room temperature (15-25°C). The unopened vial of RNase A solution is stable at room temperature. Once the vial is opened, it should be stored at -20°C. Following the addition of RNase A Solution, the Resuspension Solution should be stored at 4°C and is stable for 6 months. It is recommended to store columns at 4°C for longer periods.

Notes. Close the bag with GeneJET Endo-free Maxi Purification Columns tightly after each use! Any precipitate that forms in the buffers during storage can be redissolved by incubating briefly at 37°C, then cooling to room temperature before use.

DESCRIPTION

The GeneJET Endo-free Plasmid Maxiprep Kit is designed for large scale isolation of high quality endotoxin-free plasmid DNA from recombinant *E. coli* cultures. The kit utilizes silica-based membrane technology in the form of convenient spin columns. The kit is designed to reduce endotoxin level to <0.1 EU/μg plasmid DNA, and each prep recovers up to 1000 μg of high copy plasmid DNA that can be used in a wide variety of molecular biology procedures such as restriction endonuclease digestion, ligation, transformation, automated sequencing, *in vitro* transcription and transfection of sensitive cells (e.g. Huh7).

PRINCIPLE

Pelleted bacterial cells are resuspended and subjected to SDS/alkaline lysis to liberate plasmid DNA. The resulting lysate is neutralized to re-anneal plasmid DNA and precipitate proteins and chromosomal DNA. Cell debris and SDS precipitate are pelleted by centrifugation and further removed by filtration. The supernatant containing plasmid DNA is loaded onto the purification column. The high salt concentration of the lysate creates appropriate conditions for plasmid DNA binding to the silica membrane in the spin column. The adsorbed DNA is washed to remove contaminants and eluted with the Elution Buffer.

IMPORTANT NOTES

Buffer preparation and handling

- Add the **RNase A Solution** provided in the kit to the **Resuspension Solution** and mix thoroughly. After the addition of RNase A, the Resuspension Solution is stable for 6 months when stored at 4°C.
- If the kit will be used infrequently, divide the Resuspension Solution into an appropriate number of aliquots and supplement one aliquot with 40 µL of RNase A per 1 mL of Resuspension Solution to create a working solution. Store the remaining RNase A at -20°C. Sequential working solutions can be prepared by supplementing an aliquot of the Resuspension Solution with RNase A.
- Add the indicated volume of isopropanol to **Wash Solution I** (concentrated) and ethanol (96-100%) to **Wash Solution II** (concentrated) prior to the first use:

	10 preps (#K0861)	
	Wash Solution I	Wash Solution II
Concentrated wash solution	75 mL	75 mL
Isopropanol	25 mL	-
Ethanol (96-100%)	-	125 mL
Total volume	100 mL	200 mL

- Check the Lysis Solution and the Neutralization Solution for salt precipitation before each use. Re-dissolve any precipitate by warming the solution to 37°C, then cool back down to 25°C. Do not vigorously shake the Lysis Solution.
- Wear gloves when handling the Lysis Solution and Wash Solution I bottles as these solutions contain irritants (see p. 9 for SAFETY INFORMATION) and are harmful if they come in contact with skin, or if they are inhaled or swallowed.

ADDITIONAL MATERIALS AND EQUIPMENT REQUIRED

- Pipettes and pipette tips.
- Vortex mixer.
- Centrifuge capable of ~ 20,000 rpm (~48,000 × g) with rotor for appropriate centrifuge tubes, or centrifuge with swinging bucket rotor capable of 4,000-5,000 × g.
- Vacuum manifold (for vacuum protocol).
- Disposable gloves.
- Ethanol (96-100%).
- Isopropanol.
- Endotoxin-free 50 ml collection tubes.

Bacterial strains

High quality plasmid DNA can be obtained from various *E.coli* strains including DH10B, DH5 α , XL1-Blue, JM109, JM107, TOP10.

Culture media

The GeneJET Endo-free Plasmid Maxiprep purification protocol outlined in this manual is optimized for use with bacterial cultures grown in Luria-Bertani (LB) medium to a cell density of approximately 2-3 (OD₆₀₀ = 2-3). The use of rich growth media is not recommended.

Bacterial culture growth

- To prepare the bacterial culture, pick a single colony from a freshly streaked selective plate to inoculate 1-5 mL of LB medium supplemented with the appropriate antibiotic. Incubate for approximately 8 hours at 37°C while shaking at 200-250 rpm.
- Dilute the starter culture from 1:1,000 to 1:10,000 in LB medium and incubate for 12-16 hours (overnight) at 37°C while shaking at 200-250 rpm. The recommended bacterial culture optical density for plasmid DNA isolation is approximately 2-3 (OD₆₀₀ = 2-3).
- Harvest cells by centrifugation at 5,000 × g for 10 min. Discard the supernatant.
- The bacterial pellet can be used immediately or stored at -20°C.

Culture volume

Generally, 350 mL of overnight bacterial culture grown in LB medium is sufficient for good yield of high-copy and low-copy number plasmid DNA. However, it is important not to exceed the recommended cell mass (culture volume × OD₆₀₀), because it may decrease the quality of isolated DNA. The maximum culture volume to use can be determined using the formula below:

$$\text{Maximum culture volume (mL)} = 750/\text{OD}_{600}$$

Table 1. Copy numbers of various vectors

High-copy 300-700 copies per cell	Low-copy 10-50 copies per cell	Very low-copy Up to 5 copies per cell
pUC vectors pBluescript vectors pGEM vectors pTZ vectors pJET vectors	pBR322 and derivatives pACYC and derivatives	pSC101 and derivatives

PROTOCOL SELECTION GUIDE

The GeneJET Endo-free Plasmid Maxiprep Kit provides optimized protocols for plasmid DNA purification using low speed and high speed centrifuges, as well as using vacuum manifolds.

The following selection guide summarizes available protocols depending on the equipment used:

Equipment	Centrifugation protocol	Vacuum protocol
Low speed centrifuge (up to 5,000 x g)	Protocol A page 5	Protocol C page 7
High speed centrifuge (up to 48,000 x g)	Protocol B page 6	Protocol C page 7

PROTOCOLS

Protocol A. Plasmid DNA purification using low speed centrifuges

Step	Procedure
1	Grow up to 350 mL of bacterial culture to an OD ₆₀₀ of 2-3 as outlined on p.4. For best results calculate the maximum volume of cell culture to use by referring to the “Culture volume” on p.4. Harvest the cells by centrifugation for 10 min at 5,000 × g. Discard the supernatant.
2	Resuspend pelleted cells in 7 mL of Resuspension Solution . The bacterial pellet can be resuspended by vortexing or pipetting up and down until no cell clumps remain. Note. Ensure that the RNase A Solution has been added to the Resuspension Solution as described on p.3.
3	Add 7 mL of Lysis Solution and mix gently by inverting the tube 4-6 times until the solution becomes viscous and slightly clear. Incubate for 3 min at room temperature. Note. Do not vortex to avoid shearing chromosomal DNA. Do not incubate for more than 3 min to avoid denaturation of supercoiled plasmid DNA.
4	Add 7 mL of Neutralization Solution and mix immediately by inverting the tube 5-8 times.
5	Add 1 mL of the Endotoxin Binding Reagent and mix immediately by inverting the tube 5-8 times. Incubate 10 min at room temperature. Note. After the addition of the Neutralization Solution and Endotoxin Binding Reagent it is important to mix the contents of the tube gently, but thoroughly, to avoid localized precipitation of bacterial cell debris. The neutralized bacterial lysate should appear cloudy and contain white precipitate.
6	Add 4 mL of isopropanol. Mix immediately by inverting the tube 5-6 times.
7	Centrifuge for 20 min at 4,000-5,000 × g to pellet cell debris and chromosomal DNA. Note. Use recommended centrifugation speed.
8	Transfer the supernatant to a GeneJET Maxi Endo-free Filtration Column (white-capped) pre-assembled with collection tube (provided) by decanting or pipetting. Be careful not to overfill the column. Avoid disturbing or transferring the white precipitate. Centrifuge for 2 min at 500 × g in a swinging bucket rotor. Discard the filtration column. Note. Close the bag with columns tightly after use!
9	Add 8 mL of isopropanol. Mix immediately by inverting the tube 5-6 times (see p. 7 to continue with vacuum protocol, if preferred).
10	Transfer part of the sample (~ 20 mL) to the GeneJET Maxi Purification Column (blue-capped). Do not overfill the column. Centrifuge for 3 min at 2,000 × g in a swinging bucket rotor. Discard the flow-through and place the column back into the same collection tube. Note. Close the bag with columns tightly after use!
11	Repeat step 10 to process any remaining lysate through the purification column.
12	Add 8 mL of Wash Solution I (with isopropanol added as described on p.3) to the purification column. Centrifuge for 2 min at 3,000 × g in a swinging bucket rotor. Discard the flow-through and place the column back into the same collection tube.
13	Add 16 mL of Wash Solution II (with ethanol added as described on p.3) to the purification column. Centrifuge for 2 min at 3,000 × g in a swinging bucket rotor. Discard the flow-through and place the column into the same collection tube.
14	Centrifuge for 5 min at 3,000 × g in a swinging bucket rotor to remove residual wash solution. Discard the collection tube containing the flow-through.
15	Transfer the column to a fresh endotoxin-free 50 mL collection tube (not provided). Add 1 mL of Elution Buffer to the centre of the purification column membrane. Incubate for 2 min at room temperature and centrifuge for 5 min at 3,000 × g to elute plasmid DNA. Note. To increase the concentration of eluted DNA the volume of the Elution Buffer can be reduced to 0.7 mL . Be aware that lower volumes of Elution Buffer will decrease the overall yield of eluted DNA. To increase the overall DNA yield by 20-30% an additional elution step (optional) with Elution Buffer (0.5 mL) may be used.
16	Discard the purification column. Use the purified plasmid DNA in downstream applications or store at -20°C.

Protocol B. Plasmid DNA purification using high speed centrifuges.

Step	Procedure
1	Grow up to 350 mL of bacterial culture to an OD ₆₀₀ of 2-3 as outlined on p.4. For best results calculate the maximum volume of cell culture to use by referring to the “Culture volume” on p.4. Harvest the cells by centrifugation for 10 min at 5,000 × g. Discard the supernatant.
2	Resuspend pelleted cells in 7 mL of Resuspension Solution . The bacterial pellet can be resuspended by vortexing or pipetting up and down until no cell clumps remain. Note. Ensure that the RNase A Solution has been added to the Resuspension Solution as described on p.3.
3	Add 7 mL of Lysis Solution and mix gently by inverting the tube 4-6 times until the solution becomes viscous and slightly clear. Incubate for 3 min at room temperature. Note. Do not vortex to avoid shearing chromosomal DNA. Do not incubate for more than 3 min to avoid denaturation of supercoiled plasmid DNA.
4	Add 7 mL of Neutralization Solution and mix immediately by inverting the tube 5-8 times.
5	Add 1 mL of the Endotoxin Binding Reagent and mix immediately by inverting the tube 5-8 times. Incubate 10 min at room temperature. Note. After the addition of the Neutralization Solution and Endotoxin Binding Reagent it is important to mix the contents of the tube gently, but thoroughly, to avoid localized precipitation of bacterial cell debris. The neutralized bacterial lysate should appear cloudy and contain white precipitate.
6	Centrifuge for 10 min at 20,000 rpm (48,000 × g) to pellet cell debris and chromosomal DNA. Note. Use recommended centrifugation speed. Do not add isopropanol before centrifugation!
7	Transfer the supernatant to a GeneJET Endo-free Maxi Filtration Column (white-capped) pre-assembled with collection tube (provided) by decanting or pipetting. Be careful not to overfill the column. Avoid disturbing or transferring the white precipitate. Centrifuge for 2 min at 500 × g in a swinging bucket rotor. Discard the filtration column. Note. Close the bag with columns tightly after use!
8	Add 9 ml of isopropanol. Mix immediately by inverting the tube 5-6 times. (see p. 7 to continue with vacuum protocol, if preferred).
9	Transfer part of the sample (~ 20 mL) to the GeneJET Endo-free Maxi Purification Column (blue-capped). Do not overfill the column. Centrifuge for 3 min at 2,000 × g in a swinging bucket rotor. Discard the flow-through and place the column back into the same collection tube. Note. Close the bag with columns tightly after use!
10	Repeat step 9 to process any remaining lysate through the purification column.
11	Add 8 mL of Wash Solution I (with isopropanol added as described on p.3) to the purification column. Centrifuge for 2 min at 3,000 × g in a swinging bucket rotor. Discard the flow-through and place the column back into the same collection tube.
12	Add 16 mL of Wash Solution II (with ethanol added as described on p.3) to the purification column. Centrifuge for 2 min at 3,000 × g in a swinging bucket rotor. Discard the flow-through and place the column into the same collection tube.
13	Centrifuge for 5 min at 3,000 × g in a swinging bucket rotor to remove residual wash solution. Discard the collection tube containing the flow-through.
14	Transfer the column to a fresh endotoxin-free 50 mL collection tube (not provided). Add 1 mL of Elution Buffer to the centre of the purification column membrane. Incubate for 2 min at room temperature and centrifuge for 5 min at 3,000 × g to elute plasmid DNA. Note. To increase the concentration of eluted DNA the volume of the Elution Buffer can be reduced to 0.7 mL . Be aware that lower volumes of Elution Buffer will decrease the overall yield of eluted DNA. To increase the overall DNA yield by 20-30% an additional elution step (optional) with Elution Buffer (0.5 mL) may be used.
15	Discard the purification column. Use the purified plasmid DNA in downstream applications or store at -20°C.

Protocol C. Plasmid DNA purification using vacuum manifold

Step	Procedure
1	Perform cell collection, lysis and lysate clearing steps according to steps 1 - 9 in Protocol A on page 5 or steps 1 - 8 in Protocol B on page 6.
2	Prepare the vacuum manifold according to the supplier's instructions. Place the GeneJET Endo-free Maxi Purification column(s) (blue-capped) onto the manifold.
3	Transfer part of the sample (~ 20 mL) to the GeneJET Endo-free Maxi Purification Column (blue-capped). Be careful not to overfill the column. Apply vacuum to draw the solution through the column. Switch off the vacuum after the solution has passed through the column. Note. Close the bag with columns tightly after use!
4	Repeat step 3 to process the remaining lysate through the purification column.
5	Add 8 mL of Wash Solution I (diluted with isopropanol as described on p.3) to the purification column. Apply the vacuum to draw the solution through the column. Switch off the vacuum after the solution has passed through the column.
6	Add 16 mL of Wash Solution II (diluted with ethanol as described on p.3) to the purification column. Apply the vacuum to draw the solution through the column. Switch off the vacuum after the solution has passed through the column.
8	To dry the column, transfer the GeneJET Endo-free Maxi Purification Column into a fresh 50 mL collection tube (provided) and centrifuge for 5 min at 3,000 × g in a swinging bucket rotor.
9	Transfer the column to a fresh endotoxin-free 50 mL collection tube (not provided). Add 1 mL of Elution Buffer to the centre of the column membrane. Incubate the column for 2 min at room temperature and centrifuge for 5 min at 3,000 × g in a swinging bucket rotor to elute plasmid DNA. Note. To increase the concentration of eluted DNA volume of the Elution Buffer can be reduced to 0.7 mL . Be aware that lower volumes of Elution Buffer will decrease the overall yield of eluted DNA. To increase the overall DNA yield by 20-30% an additional elution step (optional) with Elution Buffer (0.5 mL) may be used.
10	Discard the purification column. Use the purified plasmid DNA in downstream applications or store DNA at -20°C.

TROUBLESHOOTING

Problem	Possible cause and solution
Low plasmid DNA yield	<p>Incomplete bacterial cell lysis. It is essential that the cell pellet is completely resuspended in the Resuspension Solution prior to lysis. Cell clumps should not be visible before the addition of Lysis Solution. Check the Lysis Solution for salt precipitation before each use. Redissolve any precipitate by warming the solution to 37°C, then mix well and cool to 25°C before use. Do not use more biomass than recommended. Refer to the “Culture volume” on p.4.</p> <p>Isopropanol was not added to Wash Solution I. Ensure that isopropanol was added to Wash Solution I before the first use. Follow the instructions to prepare Wash Solution I on p.3.</p> <p>Ethanol was not added to Wash Solution II. Ensure that ethanol was added to Wash Solution II before the first use. Follow the instructions to prepare Wash Solution II on p.3.</p> <p>Filtration column clogs during the filtration procedure. Reduce the volume of cell culture biomass processed per column. Avoid transferring pelleted cell debris to the purification column when loading the lysate to the column.</p> <p>Suboptimal cell culture volume. Calculate the maximum culture volume using the following formula: Maximum culture volume (mL) = 750/OD₆₀₀.</p> <p>Old Bacterial culture. Prepare a new starter culture by inoculating a freshly-isolated single bacterial colony in antibiotic-containing growth medium and grow bacteria as described in the “Bacterial Culture Growth” section on p.4.</p> <p>Inappropriate centrifuge speed. Please make sure that the recommended centrifuge speed was used during purification. It is very important to meticulously adhere to the centrifugation protocol.</p>
Suboptimal A₂₆₀/A₂₈₀ ratio	<p>Purification of plasmid DNA is not efficient. Reduce volume of cell culture. Follow recommendations described in “Culture volume”, p.4.</p>
Genomic DNA contamination	<p>Samples vigorously vortexed or shaken during cell lysis or neutralization steps. To avoid genomic DNA contamination, mix the solution (steps 3 and 4) by gently inverting the tubes 4-8 times. Do not allow the cell lysis step (step 3) to proceed for more than 3 min. Do not cultivate cells longer than 16 h in LB media. Residual genomic DNA can be removed from purified plasmid DNA using T7 DNA Polymerase (#EP0081).</p>
RNA contamination	<p>RNase A Solution was not added to the Resuspension Solution before the first use. Ensure that the RNase A Solution was added to the Resuspension Solution as described on p.3.</p>
Purified prep contains additional plasmid forms	<p>Plasmid DNA denatured during cell lysis. Denatured plasmid DNA migrates ahead of supercoiled DNA and is not suitable for enzymatic manipulations such as restriction digestion. To avoid denaturation, do not lyse the cells (step 3) for more than 3 min.</p>
Inhibition of downstream enzymatic reactions	<p>Purified plasmid DNA contains ethanol. Dry the purification column sufficiently prior to the DNA elution (steps 13 or 14 of centrifugation protocols or step 8 of vacuum protocol).</p>
No or low yields of <i>in vitro</i> transcription reaction products	<p>Purified plasmid DNA contains RNase A. If the purified DNA is used for <i>in vitro</i> transcription, linearized plasmid DNA can be repurified using the GeneJET PCR Purification Kit (#K0701) or by phenol/chloroform extraction.</p>

References

1. H.C. Birnboim, J. Doly, A rapid alkaline lysis procedure for screening recombinant plasmid DNA. *Nucleic Acids Res.* **7**, 1513 -1522 (1979).
2. B. Vogelstein, D. Gillespie, Preparative and analytical purification of DNA from agarose. *Proc. Natl. Acad. Sci. USA* **76**, 615-619 (1979).

SAFETY INFORMATION



Lysis Solution

Danger

Hazard statements:

H314 Causes severe skin burns and eye damage.

H319 Causes serious eye irritation.

Precautionary statements:

P260 Do not breathe dust/fume/gas/mist/vapours/spray.

P303+P361+P353 IF ON SKIN (or hair): Remove/Take off immediately all contaminated clothing. Rinse skin with water/shower.

P305+P351+P338 IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do.

Continue rinsing.

P310 Immediately call a POISON CENTER or doctor/physician.

P405 Store locked up.

P501 Dispose of contents/container in accordance with local/regional/national/ international regulations.



Neutralization solution

Warning

Hazard statements:

H315 Causes skin irritation.

H319 Causes serious eye irritation.

Precautionary statements:

P280 Wear protective gloves/protective clothing/eye protection/face protection.

P305+P351+P338 IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do.

Continue rinsing.

P321 Specific treatment (see on this label).

P362 Take off contaminated clothing and wash before reuse.

P332+P313 If skin irritation occurs: Get medical advice/attention.

P337+P313 If eye irritation persists: Get medical advice/attention.



Wash Solution I (concentrated)

Warning

Hazard statements:

H302 Harmful if swallowed.

H315 Causes skin irritation.

H319 Causes serious eye irritation.

Precautionary statements:

P280 Wear protective gloves/protective clothing/eye protection/face protection.

P305+P351+P338 IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do.

Continue rinsing.

P321 Specific treatment (see on this label).

P362 Take off contaminated clothing and wash before reuse.

P301+P312 IF SWALLOWED: Call a POISON CENTER or doctor/physician if you feel unwell.

P501 Dispose of contents/container in accordance with local/regional/national/international regulations.

PRODUCT USE LIMITATION

This product is developed, designed and sold exclusively for research purposes and *in vitro* use only. The product was not tested for use in diagnostics or for drug development, nor is it suitable for administration to humans or animals. Please refer to www.thermoscientific.com/onebio for Material Safety Data Sheet of the product.

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