PRODUCTINFORMATION

GenElute™ Endotoxin-free Plasmid Maxiprep Kit

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TECHNICAL BULLETIN

Product Description

Endotoxins (also known as lipopolysaccharides or LPS) are often co-purified with plasmid DNA and significantly reduce transfection efficiencies in endotoxin-sensitive cell lines. The GenElute™ Endotoxin-free Plasmid Maxiprep Kit offers a simple, rapid, cost-effective method for isolating up to 1.2 mg of endotoxin-free plasmid DNA (≤0.1 EU/µg DNA) for use in cell transfection.

Overnight recombinant *E. coli* culture is harvested by centrifugation and subjected to a modified alkaline-SDS lysis procedure. Endotoxins are removed from cleared lysate with simple extraction/phase separation steps. Plasmid DNA is further purified by absorption onto silica in the presence of high salt. After a spin-wash step, the bound plasmid DNA is eluted in endotoxin-free water.

The recovered plasmid DNA is predominately in its supercoiled form. There is no visual evidence of genomic DNA or RNA contamination by agarose gel electrophoresis. The DNA is ready for immediate use in downstream applications such as transfection, restriction endonuclease digestion, cloning, sequencing, and PCR† amplification.

Reagents Provided

Sufficient for 10 preparations

- Resuspension Solution, Product No. R1149 — 55 ml
- RNase A (20 mg/ml), Product No. R6148 — 0.3 ml
- Lysis Solution, Product No. L1912 — 55 ml
- Neutralization Solution, Product No. N2409 — 40 ml
- Endotoxin Removal Solution, Product No. E4274 — 30 ml
- DNA Binding Solution, Product No. B1555 — 40 ml
- Optional Wash Solution, Product No. W4011 — 100 ml
- Wash Solution Concentrate, Product No. W3886 — 40 ml
- Endotoxin Free Water, Product No. 210-7 — 100 ml
- GenElute™ Maxiprep Binding Columns in Tubes, Product No. G6665 — 10 each
- Collection Tubes, 50 ml, Product No. C4353 — 10 each

Equipment and Reagents Required But Not Provided
(Sigma product numbers have been given where appropriate)

- Ethanol (95-100%), Product No. E7148 or E7023
- Centrifuge and tubes capable of 15,000 X g
- Centrifuge and swinging bucket rotor capable of 3,000-5,000 X g, with adapters for Corning® or equivalent 15 ml and 50 ml conical tubes
- Centrifuge bottles, Product No. Z35,373-6
- Centrifuge tubes, Oak Ridge, Product No. T2918
- Centrifuge tubes, Corning® or equivalent 15 and 50 ml conical, Product Nos. C3048, C8046,and C8171
- 37°C water bath

Precautions and Disclaimer

The GenElute Endotoxin-free Plasmid Maxiprep Kit is for laboratory use only. Not for drug, household or other uses. DNA Binding Solution and Optional Wash Solution contain guanidine, which is harmful. Wear gloves, safety glasses, and suitable protective clothing when handling these solutions or any reagent provided in the kit. See the Material Safety Data Sheet (MSDS).

Storage

Store the kit at room temperature. If any kit reagent forms a precipitate upon storage, warm at 65°C until dissolved, and allow to cool to room temperature before use. The Endotoxin Removal Solution (E4274) can be stored at room temperature or at -20°C. but should be cooled to 4°C or lower before use.

Preparation Instructions

1. Resuspension Solution: Briefly centrifuge the RNase A tube. Add 275 µl of RNase A to Resuspension Solution and mix the solution thoroughly prior to initial use.

2. Wash Solution: Add 160 ml of 95-100% ethanol to Wash Solution Concentrate prior to initial use. Tightly cap the bottle after each use to prevent the evaporation of ethanol.

3. Mix the Endotoxin Removal Solution (E4274) briefly and incubate the bottle on ice for >10 minutes before use.
4. Water bath: Heat water bath to 37°C.

Procedure

Note: A swinging bucket rotor is necessary for steps 5-9 of this procedure. These centrifugations may be performed at room temperature.

1. **Harvest cells.** Pellet 25-130 ml of overnight bacterial culture in a centrifuge bottle by centrifuging at 3,000-5,000 X g for 10 minutes. Remove all the media supernatant.

   Note: The maximum recommended culture volume is 130 ml for LB (Luria Broth) and 65 ml for rich media such as TB (Terrific Broth) and 2xYT.

2. **Resuspend cells.** Completely resuspend the bacterial pellet with 4.8 ml of Resuspension Solution by pipetting up and down. Make sure the cells are completely resuspended until homogenous. Incomplete resuspension will result in poor recovery. Transfer the suspension into a centrifuge tube capable of ≥15,000 X g (Oak Ridge style or equivalent).

   Note: Prior to first time use, be sure to add the appropriate amount of RNase A to the Resuspension Solution. See Preparation Instructions.

3. **Lyse cells.** Lyse the resuspended cells by adding 4.8 ml of Lysis Solution. Immediately mix the contents by gentle inversion 6-8 times. Incubate at room temperature until a clear viscous mixture is formed. **Do not allow the lysis reaction to exceed 5 minutes.** Prolonged alkaline lysis may permanently denature supercoiled plasmid DNA, rendering it unsuitable for most downstream applications.

   Note: **Do not vortex** the cells during lysis. Harsh mixing will shear genomic DNA, resulting in chromosomal DNA contamination in the final recovered plasmid DNA.

4. **Neutralize.** Add 3.2 ml of Neutralization Solution to the lysate. Immediately mix the contents thoroughly by gentle inversion. Pellet the cell debris by centrifuging at ≥15,000 X g for 15 minutes at 2-8°C. Transfer the cleared lysate into a Corning® or equivalent 15 ml conical tube.

   Note: Make sure the lysate is thoroughly mixed after addition of Neutralization Solution. Do not vortex; this will result in shearing of chromosomal DNA. If the supernatant contains a large amount of floating cell debris after centrifugation, re-centrifuge the supernatant before proceeding to step 5.

5. **Remove endotoxin**

   a. Add 1.2 ml of ice-cold Endotoxin Removal Solution to the lysate. Mix thoroughly by inversion for 1 minute. Chill the tube on ice for ≥10 minutes. Mix 2-3 times during the ice incubation. The solution should be light blue and clear.

   b. Warm the tube in a 37°C water bath for 5 minutes. The solution will turn cloudy. To separate the phases, centrifuge the tube at 3,000-5,000 x g in a swinging bucket rotor for 5 minutes at room temperature. The clear upper phase contains plasmid DNA. The blue lower phase contains endotoxins.

   c. Carefully transfer the clear upper phase into a fresh 15 ml conical tube. Discard the blue lower phase.

   d. Repeat steps a and b, then continue to step 6.

6. **Bind DNA.** Carefully transfer the clear upper phase into a fresh 50 ml tube. Add 3.2 ml of DNA Binding Solution to the endotoxin-free lysate. Mix the contents thoroughly by inversion or vortexing. Load the lysate onto a GenElute Maxiprep binding column seated in a collection tube. Close the cap and centrifuge in a swinging bucket rotor at 3,000-5,000 X g for 2 minutes.

7. **Optional wash (use only with *EndA*$^+$ strains).** Discard the flow-through and add 8.0 ml of Optional Wash Solution to the column. Centrifuge in a swinging bucket rotor at 3,000-5,000 X g for 5 minutes. Discard the flow-through.

   Note: When working with bacterial strains containing the wild-type EndA$^+$ gene, such as HB101, JM101, and the NM and PR series, the Optional Wash Step is necessary to avoid nuclease contamination in the final plasmid prep.

8. **Wash column.** Add 15 ml of Wash Solution to the column. Close the cap and centrifuge in a swinging bucket rotor at 3,000-5,000 X g for 5 minutes.

Note: Prior to first time use, be sure to add ethanol to the Wash Solution Concentrate. See Preparation Instructions. Make sure Wash Solution is completely removed from the column before proceeding to step 9.

9. **Elute DNA.** Transfer the binding column to a fresh collection tube. Add 5 ml of Endotoxin Free Water to the column. Close the cap and centrifuge in a swinging bucket rotor at 3,000-5,000 X g for 3-5 minutes. If a more concentrated plasmid DNA prep is required, the elution volume may be reduced by half; however, this may result in a reduction in the total plasmid DNA yield.

The DNA is now present in the eluate and is ready for immediate use or storage at -20°C. To prevent introducing endotoxins into the purified plasmid DNA, use only new, unhandled plasticware, which is considered to be endotoxin-free.

Results

Recovery and purity of the plasmid DNA may be determined by spectrophotometric analysis. The ratio of absorbance at 260 nm to 280 nm should be 1.7 to 1.9. Size and quality of DNA may be determined by agarose gel electrophoresis or pulsed field electrophoresis.

Troubleshooting Guide

Problem	Cause	Solution
Poor or low plasmid DNA yield	Wash Solution is too concentrated	Confirm the Wash Solution Concentrate was diluted with the specified volume of ethanol. Keep bottle tightly capped between uses to prevent evaporation.
	Number of cells is insufficient	• Culture may be too old. Prepare a new culture. • Confirm cell density. Grow culture to 2.5-3.0 OD_{600}
	Plasmid replication is poor	Confirm cells were grown in appropriate media under optimized conditions.
	Antibiotic activity is insufficient	Use fresh antibiotic for growth of overnight cultures. Most antibiotics are light-sensitive and degrade during long term storage at 2-8°C.
	Alkaline lysis is prolonged	Reduce the time for step 3, cell lysis, to 3 minutes or until the suspended cells form a clear viscous mixture after inversion with the Lysis Solution.
	Residual supernatant from cell media	After initial centrifugation of cell culture, remove supernatant and centrifuge a second time to remove any remaining supernatant.
	Precipitation of cell debris is incomplete	Reduce the initial volume of cell culture.
	Lysis is incomplete	Reduce the initial volume of cell culture or increase the lysis time (step 3) while monitoring the lysis visually.
O.D. of final product does not match actual quantity of plasmid	Wash Solution is diluted with ethanol containing impurities	Check the absorbance of ethanol between 250 and 300 nm. Do not use ethanol with high absorbance. Traces of impurities may remain on binding column after washing. Impurities may show up in the eluate and may contribute to the absorbance in the final product.
	RNA contamination because RNase A treatment is insufficient	Confirm that RNase A was added to the Resuspension Solution prior to first use. RNase A may degrade at high temperatures (>65°C) or prolonged storage (>6 months at room temperature).
	Chromosomal DNA contamination due to shearing	Do not vortex or vigorously shake the cells during lysis (step 3) or neutralization (step 4).
	Chromosomal DNA contamination due to overgrown culture	Do not use cultures that have grown for more than 24 hours or are in the cell death phase.

Troubleshooting Guide (continued)

Problem	Cause	Solution
Poor performance in downstream enzymatic applications	Purification is incomplete	Salts in one or more of the solutions may have precipitated. Heat the solution at 65°C until dissolved. Cool to room temperature prior to use.
	DNA concentration is too low	• Precipitate the DNA with ethanol, then resuspend the DNA in a smaller volume of Endotoxin Free Water. **Or** • Elute silica-bound DNA with less Endotoxin Free Water. Note that using less water may reduce the overall recovery.
	DNA was prepared from $EndA^+$ strains	The Optional Wash Step (step 7) must be included when recovering DNA from $EndA^+$ strains.
	The final plasmid DNA eluate contains too much salt	Precipitate the DNA using ethanol. Dry the pellet. Redissolve in Endotoxin Free Water.
	Column contains residual ethanol from diluted Wash Solution	Re-centrifuge the column for 1 minute after washing (step 8) to remove any residual Wash Solution.
Poor A_{260}/A_{280} ratios for the purified DNA	Purification is incomplete due to column overloading	Reduce the initial volume of cell culture.
Residual endotoxin (>0.1 EU/µg DNA)	Culture overgrown or too much culture used	Grow culture 12-16 hours with vigorous shaking. Do not exceed the recommended maximum culture volume (130 ml).
	Carryover of the endotoxin-enriched lower (blue) phase	Avoid pipetting any part of the blue lower phase when transferring the clear upper phase in step 5.
The Endotoxin Removal Solution is in two phases	Storage temperature is higher than 25°C	Mix the solution briefly and incubate on ice for >10 minutes before use. Solution will be clear, blue and homogeneous (in one phase).
Additional forms of the plasmid present (single-stranded DNA)	Plasmid DNA is permanently denatured	There will be a second band ahead of supercoiled DNA during electrophoresis. Do not allow the lysis reaction (step 3) to exceed 5 minutes. Note that nicked (covalently open) double-stranded plasmid DNA runs slower than supercoiled DNA during electrophoresis.

Related Products	Product No.
Water, Molecular Biology Grade	W4502
LB Broth, EZMix™	L7658
LB Agar, EZMix™	L7533
Terrific Broth, EZMix™	T9179
Precast Agarose Gels, 1.0%, 8 well	P5472
TAE Buffer (10X)	T9650
Gel Loading Solution	G2526
DirectLoad™ Wide Range DNA markers, 50-10,000 bp	D7058
Ethidium bromide, Aqueous Solution,10 mg/ml	E1510
GenElute Endotoxin-free Plasmid Midiprep Kit	PLED25

Related Products	Product No.
TBE Buffer (10X)	T4415
β-Galactosidase Reporter Gene Activity Detection Kit	GAL-A
β-Galactosidase Fluorescence Activity Detection Kit	GAL-F
Chloramphenicol Acetyltransferase Reporter Gene Activity Detection Kit	CAT-A
Luciferase Assay Kit	LUC-1
Escort III Transfection Reagent	L3037
Escort IV Transfection Reagent	L3287

[†]The PCR process is covered by patents owned by Hoffman-LaRoche, Inc.