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ProductInformation

GenElute™ Plasmid Maxiprep Kit

Product Numbers **PLX10, PLX50**
Technical Bulletin No. MB-675
July 2000

TECHNICAL BULLETIN

Product Description
The GenElute™ Plasmid Maxiprep Kit offers a simple, rapid, cost-effective method for isolating plasmid DNA from recombinant *E. coli* cultures. By combining silica-based membrane technology and the convenience of a spin column format, up to 1.2 mg of plasmid DNA can be recovered from 180-200 ml of LB broth or 100 ml of Terrific Broth in about 45 minutes.

Overnight recombinant *E. coli* culture is harvested with centrifugation and subjected to a modified alkaline-SDS lysis procedure followed by adsorption of the DNA onto silica in the presence of high salts[1,2]. Contaminants are then removed by a simple spin-wash step. Finally, the bound DNA is eluted in water or Tris-EDTA buffer.

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

Reagents Provided	Product No.	PLX10 10 Preps	PLX50 50 Preps
Resuspension Solution (S1)	R1149	65 ml	350 ml
Ribonuclease A	R6148	0.4 ml	2 ml
Lysis Solution (S2)	L1912	65 ml	350 ml
Neutralization/Binding Buffer (S3)	N5158	90 ml	425 ml
Optional Wash Solution (OWS)	W4011	90 ml	425 ml
Wash Solution Concentrate (WS)	W3886	31 ml	2 x 75 ml
Elution Solution (ES, 10 mM Tris-HCl, 1 mM EDTA, pH approx. 8.0)	E5650	60 ml	280 ml
GenElute™ Maxiprep Binding Columns in Tubes	G6665	10 each	5 x 10 each
Collection Tubes, 50 ml capacity	C4353	10 each	5 x 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 with swinging bucket rotor capable of 3000-5000 x g
- Centrifuge capable of 12,000-15,000 x g
- Centrifuge bottles, 250 ml, Product No. Z35,373-6
- Centrifuge tubes, Oak Ridge, Product No. T2918

Precautions and Disclaimer
The GenElute Plasmid Maxiprep Kit is for laboratory use only. Not for drug, household or other uses. Neutralization/Binding Buffer and Optional Wash Solution contain guanidine, which is harmful. Wear gloves, safety glasses, and suitable protective clothing when handling these solutions or any reagents provided with 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 the precipitate dissolves. Allow the reagent to cool to room temperature before using.

Preparation Instructions

1. Wash Solution (WS): Dilute the Wash Solution Concentrate with 124 ml (10 reaction package) or 600 ml (300 ml in each of 2, in 50 reaction package) of 95-100% ethanol prior to initial use. After each use, tightly cap the diluted wash solution to prevent the evaporation of ethanol.
2. Resuspension Solution (S1): Spin the tube of RNase solution (Product No. R6148) briefly to collect the solution in the bottom of the tube. Add 330 μl (10 reaction package) or 1.65 ml (50 reaction package) RNase A to the Resuspension Solution (S1) prior to initial use.

Procedure

All steps are carried out at room temperature. If centrifuges/rotors for the required g-forces are not available, use the maximum g-force possible and increase the spin time. A swinging bucket rotor is necessary for steps 5-8.

1. **Harvest cells.** Pellet 25-200 ml overnight recombinant *E. coli* culture by centrifuging at 3,000-5,000 X g for 5-10 minutes. Discard the media supernatant.

   Note: The maximum culture volume capacity for the GenElute Maxiprep binding column is 200 ml for LB (Luria broth) and 100 ml for rich media such as TB (terrific broth) and 2xYT.

2. **Resuspend cells.** Completely resuspend the bacterial pellet with 6.0 ml Resuspension Solution (S1). Vortex or pipet up and down to thoroughly resuspend the cells. Make sure the cells are completely resuspended to a homogenous suspension. Incomplete resuspension will result in poor recovery.

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

3. **Lyse cells.** Lyse the resuspended cells by adding 6.0 ml Lysis Solution (S2). Gently invert the tube 8-10 times to mix until the mixture becomes clear and viscous. You may need to incubate at room temperature for 1-2 minutes. For larger culture volumes (>100 ml), incubate at room temperature for 3-5 minutes. Do not allow the lysis reaction to exceed 5 minutes. Prolonged alkaline lysis may permanently denature supercoiled plasmid DNA that may render 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.** Precipitate the cell debris by adding 8.0 ml Neutralization/Binding Buffer (S3). Gently invert the tube 4-6 times. Pellet the cell debris by centrifuging at 12,000-15,000 X g for 10 minutes.

   Note: Cell debris, proteins, lipids, SDS and chromosomal DNA should fall out of solution as a cloudy, viscous precipitate upon addition of S3. If the supernatant contains a large amount of floating particulates after centrifugation, re-centrifuge the supernatant before proceeding to step 5.

5. **Load cleared lysate.** Insert a GenElute Maxiprep binding column into a 50 ml collection tube, if not already assembled. Transfer the cleared lysate from step 4 to the column, and centrifuge at 3,000-5,000 X g for 1-2 minutes. Discard flow-through liquid.

   Note: A swinging bucket rotor is necessary for this and all centrifugation steps that follow.

6. **Optional wash (use only for $EndA^+$ strains).** Add 8.0 ml Optional Wash Solution (OWS) to the GenElute Maxiprep binding column. Centrifuge in a swinging bucket rotor at 3,000-5,000 X g for 5 minutes. Discard flow-through liquid.

   Note: When working with bacterial strains containing the wild-type $EndA^+$ gene, such as HB101, JM109, and the NM and PR series, the Optional Wash step is necessary to avoid nuclease contamination of the final plasmid DNA product.

7. **Wash column.** Add 15 ml diluted Wash Solution (WS) to the GenElute Maxiprep binding column. Centrifuge in a swinging bucket rotor at 3,000-5,000 X g for 5 minutes. Discard the flow-through liquid and centrifuge again at maximum speed for 2 minutes without any additional Wash Solution to remove excess ethanol.

   Note: Prior to first time use, be sure to add ethanol to the concentrated Wash Solution (WS). See Preparation Instructions. The column wash step removes residual salt and other contaminants introduced during the column load.

8. **Elute DNA.** Transfer the GenElute Maxiprep binding column to a fresh 50 ml collection tube. Add 5 ml of Elution Solution (ES) or molecular biology grade water to the column. For DNA sequencing and other enzymatic applications, use water or 5 mM Tris-HCl, pH 8.0, as an eluant. Centrifuge in a swinging bucket rotor at 3,000-5,000 X g for 5 minutes. If a more concentrated plasmid DNA prep is required, reduce the elution volume to a minimum of 1 ml. Total DNA recovered will be less than if a 5 ml elution volume is used.

Results

The DNA is now present in the eluate and is ready for immediate use or storage at -20°C. Recovery and purity may be determined by spectrophotometric analysis. The ratio of absorbance at 260 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.

Note: If floating particulates are evident during the modified alkaline-lysis centrifugation steps (steps 1 and 4), or if eluate volumes are considerably lower than load/wash volumes applied during GenElute Maxiprep binding column centrifugations, increase the centrifuge time.

Troubleshooting Guide

Problem	**Cause**	**Solution**
Poor or low recovery	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.
	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 solution after inversion with the Lysis Solution.
	Residual supernatant from cell media	After initial centrifugation step 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 the ethanol between 250 and 300 nm. Do not use denatured ethanol with high absorbance. Traces of impurities may remain on binding column after washing and 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 due to high temperatures (>65°C) or prolonged storage (>6 months).
	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.
Poor A_{260}/A_{280} ratios for the purified DNA	Purification is incomplete due to high quantity of DNA	Reduce the initial volume of cell culture.

Troubleshooting Guide (cont.)

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 water or Elution Solution **Or** • Elute silica-bound DNA with less Elution Solution. Note that using less Elution Solution may reduce the overall recovery.
	DNA was prepared from $EndA^+$ strains	The Optional Wash Step (step 6) 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 water or Elution Solution. Note that Elution Solution contains EDTA, which may chelate divalent cations (e.g. Mg^{++}) which are important co-factors for many enzymes.
	The column contains residual ethanol from the diluted Wash Solution	Re-centrifuge the column for 1 minute after the washing (step 7) to remove any residual Wash Solution.
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.

References

1. Birnboim, H.C., and Doly, J., Nucleic Acids Res., **7**, 1513-1522 (1979)
2. Vogelstein, B., and Gillespie, D., Proc. Natl. Acad. Sci. USA, **76**, 615-619 (1979)

Related Products	**Product No.**
Molecular Biology Grade Water	W4502
LB Broth, EZMix™	L7658
LB Agar, EZMix™	L7533
Terrific Broth, EZMix™	T9179
Precast Agarose Gels, 1.25%, 8 well	P5472
TAE Buffer (10X)	T9650
TBE Buffer (10X)	T4415
Gel Loading Solution	G2526
DirectLoad™ Wide-range DNA Marker	D7058
Ethidium bromide, 10 mg/ml	E1510
GenElute Plasmid Miniprep Kits	PLN-10, PNL-70, and PLN-350
GenElute Plasmid Midiprep Kits	PLD-25, PLD-100

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

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