GENELUTE™ PLASMID MINI-PREP KIT

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Product Description
The GenElute™ Plasmid Mini-Prep 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, the Mini-Prep Kit recovers up to 20 µg of plasmid DNA from 5 ml of LB broth or 3 ml of Terrific Broth in 35-45 minutes. Unlike many other alkaline-SDS lysis mini kits and protocols, the GenElute Plasmid Mini-Prep Kit requires:

- No alcohol precipitations
- No dangerous chemicals such as phenol or chloroform
- No cumbersome steps associated with resins and magnetic slurries
- No long centrifugation or incubation steps (>10 minutes)

Overnight recombinant *E. coli* culture is harvested via 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 by agarose gel electrophoresis. The DNA is ready for immediate use in further downstream applications such as restriction digestion, ligation, sequencing, PCR[†], and transfection.

Reagents Provided
Sufficient for 50 applications

- Resuspension Solution (S1), Product No. R1149 — 11 ml
- RNase A Solution, Product No. R6148 20 mg/ml — 0.1 ml
- Lysis Solution (S2), Product No. L1912 — 11 ml
- Neutralization/Binding Solution (S3), Product No. N5158 — 20 ml
- Optional Wash Solution (OWS), Product No. W4011 — 30 ml
- Wash Solution Concentrate (WS), Product No. W3886 — 11 ml
- Elution Solution, 1X TE (ES), Product No. E5650 — 10 ml
- GenElute™ Mini Spin Columns With 2 Collection Tubes, Product No. G6415 — 50 each

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

- Ethanol (95-100%), Product No. E7148 or E7023
- Microcentrifuge
- Microcentrifuge tubes

Precautions and Disclaimer
The GenElute Plasmid Mini-Prep Spin Kit is for laboratory use only. Not for drug, household or other uses. Neutralization/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 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 44 ml of 95-100% ethanol prior to initial use. After each use tightly cap the diluted wash solution to prevent the evaporation of ethanol.

   Note: Before using denatured alcohol to dilute the Wash Solution Concentrate, check the absorbance of the ethanol between 250 and 300 nm. Do not use denatured ethanol with strong absorbance. If a strong absorbance is observed, use non-denatured ethanol for addition to the WS. Traces of the absorbing impurities may remain on the binding column after washing, which may be eluted and contribute to absorbance of the final plasmid product.

2. Resuspension Solution (S1): Add 55 µl RNase A Solution to the Resuspension Solution (S1) prior to initial use.

Procedure

All steps are carried out at room temperature.

1. Pellet 1-5 ml overnight recombinant *E. coli* culture by centrifuging at ≥12,000 X g for 1 minute. Discard the media supernatant.

   Note: For LB media and a high copy plasmid, the maximum culture volume capacity for the GenElute Mini Spin Column is 3 ml; for a low copy plasmid (e.g. pBR322) the maximum culture volume capacity is 5 ml. For rich media such as TB (terrific broth) and 2X YT, reduce the culture volume to half of the above limits.

2. Completely resuspend the bacterial pellet with 200 µl Resuspension Solution (S1). Vortex or pipet up and down to thoroughly resuspend the cells. Make sure the cells are completely resuspended to a homogenous solution. Incomplete resuspension will result in poor recovery.

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

3. Lyse the resuspended cells by adding 200 µl Lysis Solution (S2). Gently invert the tube 4-6 times or until a clear viscous solution is formed. 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. Precipitate the cell debris by adding 350 µl Neutralization/Binding Solution (S3). Gently invert the tube 4-6 times. Cell debris, proteins, lipids, SDS, and chromosomal DNA should fall out of solution in the form of a cloudy viscous precipitate upon addition of S3. Pellet the cell debris by centrifuging at ≥12,000 X g for 5-10 minutes. If the supernatant contains a large amount of floating particulates upon centrifugation, repeat the centrifugation step before proceeding to step 5.

5. Insert a GenElute Mini Spin Column into a microcentrifuge tube (not provided). Transfer the supernatant or clear lysate from step 4 to the GenElute Mini Spin Column, and centrifuge at ≥12,000 X g for 1 minute.

6. For $EndA^+$ *E. coli* strains only, discard the eluate and add 500 µl Optional Wash Solution (OWS) to the column. Centrifuge at ≥12,000 X g for 1 minute.

   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 of the final plasmid DNA product.

7. Discard eluate in the collection tube. Add 750 µl diluted Wash Solution (WS) to the column. Centrifuge at ≥12,000 X g for 1 minute. Discard the eluate and centrifuge again at maximum speed for 2 minutes without any additional Wash Solution to remove excess ethanol. The column-wash step removes residual salt and buffer contaminants introduced during the column load.

8. Transfer the GenElute Mini Spin Column to the provided collection tube. Add 100 µl 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 elution solution. Centrifuge at ≥12,000 X g for 1 minute. If a more concentrated plasmid DNA recovery is needed, reduce the elution volume down to a minimum of 50 µl. Total DNA recovered will be less than if a 100 µl 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 pulse 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 Mini Spin 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.
	Insufficient number of cells	• Culture may be too old. Prepare a new culture. • Confirm cell density.
	Poor plasmid replication	Confirm cells were grown in correct media under optimized conditions.
	Insufficient antibiotic activity	Use fresh antibiotic for growth of overnight cultures. Most antibiotics are light sensitive and degrade during long term storage at 2-8°C.
	Prolonged alkaline lysis	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.
	Incomplete precipitation of cell debris	Reduce the initial volume of cell culture.
	Incomplete lysis	Reduce the initial volume of cell culture or increase the lysis time (Step 3) while monitoring the lysis visually.
Poor 260/280 ratios for the purified DNA	Incomplete purification due to high amount of DNA	Reduce the initial volume of cell culture.
Chromosomal DNA contamination	Shearing of genomic DNA	Do not vortex or vigorously shake the cells during the lysing step (Step 3) or the neutralization step (Step 4).
	Culture was overgrown	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
RNA contamination	Insufficient RNase A treatment	Confirm the Resuspension Solution had the RNase A Solution added to it prior to first use. The RNase A Solution may degrade due to high temperatures (>65°C) or prolonged storage (>6 months).
Poor performance in downstream enzymatic applications	Incomplete purification	Salts in one or more of the buffers may have precipitated out of solution. Heat the buffer 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.
	High salt content in the final plasmid DNA eluate	Precipitate the DNA using ethanol. Use an elution buffer other than Elution Solution (ES). ES contains EDTA, which may chelate divalent cations (e.g. Mg^{++}) which are important co-factors for many enzymes.
	Residual ethanol from the Wash Solution	Re-centrifuge the column for 1 minute after the Wash Step 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., Nucl. 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
DNA markers, 50-10,000 bp	D7058
Ethidium bromide, 10 mg/ml	E1510

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

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