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ProductInformation

GenElute ä Plasmid Maxiprep Kit

Product Codes **PLX15 and PLX50**

TECHNICAL BULLETIN

Product Description

The GenElute™ Plasmid Maxiprep Kit offers a simple, rapid, and cost-effective method for isolating plasmid DNA from recombinant *E. coli* cultures. By combining silica-binding technology and the convenience of a spin column format, up to 1.2 mg of plasmid DNA can be recovered from 25–200 ml of Luria Broth (LB) in about 45 minutes. Note that actual yield and optimal volume of culture to use depend on the plasmid and the culture medium (see Procedure, step 1).

An overnight recombinant *E. coli* culture is harvested by 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 spin-wash step. Finally, the bound DNA is eluted in water or Tris-EDTA buffer.

The recovered plasmid DNA is predominantly 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 Code	PLX15 15 Reactions	PLX50 50 Reactions
Resuspension Solution	R1149	100 ml	350 ml
RNase A Solution	R6148	0.6 ml	2.0 ml
Lysis Buffer	L1912	100 ml	350 ml
Neutralization/Binding Solution	N5158	140 ml	480 ml
Column Preparation Solution	C2112	225 ml	3 X 225 ml
Optional Wash Solution	W4011	135 ml	480 ml
Wash Solution Concentrate	W3886	50 ml	2 X 80 ml
Elution Solution (10 mM Tris-HCl, pH 8.0, 1 mM EDTA)	E5650	90 ml	300 ml
GenElute Maxiprep Binding Columns in Tubes	G6665	15 each	5 x 10 each
Collection Tubes, 50 ml capacity	C4353	15 each	5 x 10 each

Equipment and Reagents Required But Not Provided

- Ethanol (95–100%), Product Code E7148, E7023, or 459836
- Centrifuge with swinging bucket rotor capable of 3,000–5,000 x *g*
- Centrifuge capable of 12,000–15,000 x *g*
- Centrifuge bottles, 250 ml, Product Code Z353736
- Centrifuge tubes, Oak Ridge, Product Code T2918

Precautions and Disclaimer

The GenElute Plasmid Maxiprep Kit is for laboratory use only, not for drug, household or other uses. The Neutralization/Binding Solution and the Optional Wash Solution contain guanidine, which is harmful. The Column Preparation Solution is an irritant. Consult the MSDS for information regarding hazards and safe handling practices.

Preparation Instructions

1. **Thoroughly mix reagents**. Examine reagents for precipitation. If any reagent forms a precipitate, warm at 55–65 °C until the precipitate dissolves. Allow the reagent to cool to room temperature before use.

2. **Resuspension Solution**: Add 500 µl (15-reaction package) or 1.75 ml (50-reaction package) of the RNase A Solution to the Resuspension Solution prior to initial use.

3. **Wash Solution**: Dilute the Wash Solution Concentrate with 95–100% ethanol prior to initial use.
   - Add 200 ml 95–100% ethanol to the concentrate bottle from the 15-reaction package.

     or
   - Add 320 ml 95–100% ethanol to each concentrate bottle from the 50-reaction package.

   After each use, tightly cap the diluted wash solution to prevent the evaporation of ethanol.

Storage

Store the kit at room temperature. If any kit reagent forms a precipitate during storage, see Preparation Instructions, above.

Procedure

All steps are carried out at room temperature.

1. **Harvest cells.** Pellet 25–200 ml of an overnight recombinant *E. coli* culture by centrifugation. The optimal volume of culture to use depends upon the culture density. For best yields, follow the instructions in the note below. Transfer the appropriate volume of the recombinant *E. coli* culture to a centrifuge bottle and pellet the cells at 3,000–5,000 x *g* for 5–10 minutes. Remove and discard all of the supernatant.

Note: For best results, start with a single colony from a freshly streaked plate. Grow in Luria broth (LB) containing the appropriate antibiotic at 37 °C with vigorous shaking (250–300 RPM) overnight. Measure the absorbance of the overnight culture at 600 nm. Use a total cell mass of approximately 300, where cell mass equals OD600 x ml of culture.

To calculate the volume of culture to use, take the desired cell mass (300) and divide by the absorbance of the overnight culture at 600 nm. For example, with a very dense culture of recombinant *E. coli* grown to an OD600 of about 4.0, use only 75 ml of the culture. With a less dense culture, where OD600 is about 2.0, use 150 ml.

For low copy plasmids, use a total cell mass of 500. A higher cell mass can cause a reduction in yield. For cultures grown in rich media, less volume may be necessary.

2. **Resuspend cells.** Prior to first time use, be sure to add the appropriate volume of the RNase A Solution to the Resuspension Solution. Completely resuspend the bacterial pellet with 6.0 ml of the Resuspension Solution by pipetting up and down. Make sure that 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).

3. **Lyse cells.** Lyse the resuspended cells by adding 6.0 ml of the Lysis Buffer. Immediately mix the contents by gentle inversion (6–8 times) until the mixture becomes clear and viscous. **Do not vortex.** Harsh mixing will shear the genomic DNA, resulting in chromosomal DNA contamination in the final recovered plasmid DNA.

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.

4. **Neutralize.** Precipitate the cell debris by adding 8.0 ml of the Neutralization/Binding Solution. Gently invert the tube 4–6 times. Pellet the cell debris by centrifuging at ≥15,000 x *g* for 10–15 minutes. Cell debris, proteins, lipids, SDS, and chromosomal DNA should fall out of solution as a cloudy, viscous precipitate. If the supernatant contains a large amount of floating cell debris after centrifugation, re-centrifuge the supernatant before proceeding to step 5.

5. **DNA Binding Column Preparation.** Insert a GenElute Maxiprep Binding Column into a 50 ml collection tube. Add 12 ml of Column Preparation Solution to each column and centrifuge in a swinging bucket rotor at 3,000–5,000 x *g* for 1-2 minutes. Discard the eluate.

Note: The Column Preparation Solution maximizes binding of DNA to the filter resulting in more consistent yields. This column preparation step can be conveniently carried out during or prior to step 4.

6. **Load cleared lysate.** Transfer the cleared lysate from step 4 to the prepared DNA Binding Column seated in a 50 ml collection tube and centrifuge at 3,000–5,000 x *g* in a swinging bucket rotor for 1–2 minutes. Discard the flow-through liquid.

7. **Optional Wash (use only for endA$^+$ strains).** Add 8.0 ml of the Optional Wash Solution to the column. Centrifuge in a swinging bucket rotor at 3,000–5,000 x *g* for 2 minutes. Discard the eluate.

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.

8. **Wash column.** Prior to first time use, be sure to add ethanol to the Wash Solution Concentrate. Add 15 ml of the diluted Wash Solution to the column. Centrifuge in a swinging bucket rotor at 3,000–5,000 x *g* for 5 minutes. The column wash step removes residual salt and other contaminants introduced during the column load. Make sure that the Wash Solution is completely removed

9. **Elute DNA.** Transfer the column to a fresh 50 ml collection tube. Add 5 ml of Elution Solution or molecular biology reagent 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 3–5 minutes. The DNA is now present in the eluate and is ready for immediate use or storage at –20 °C.

Note: If a more concentrated plasmid DNA preparation is required, the elution volume may be reduced to a minimum of 1 ml. For optimal recovery in 1 ml, preheat the Elution Solution to 65 °C and add directly to the binding column. Allow the preheated Elution Solution to soak into the binding column for 10 minutes before centrifugation. Incubating with preheated Elution Solution will improve recovery, but the total plasmid DNA yield will likely be less than with elution in the full 5 ml.

Results

Recovery and purity may be determined by spectrophotometric analysis. The ratio of absorbance at 260 nm to 280 nm (A260/A280) should be 1.7 to 1.9. Size and quality of the DNA may be determined by agarose gel electrophoresis or pulse field electrophoresis.

References

1. Birnboim, H.C., and Doly, J., A rapid alkaline extraction procedure for screening recombinant plasmid DNA. Nucleic Acids Res., **7**, 1513-1523 (1979).

2. Vogelstein, B., and Gillespie, D., Preparative and analytical purification of DNA from agarose. Proc. Natl. Acad. Sci. USA, **76**, 615-619 (1979).

Troubleshooting Guide

Problem	Cause	Solution
Poor or low plasmid DNA recovery	Binding columns were spun in a fixed angle rotor, or with insufficient *g*-force.	Binding columns must be spun in a swinging bucket rotor at 3,000–5,000 × *g* in steps 5–9 for liquids to pass through efficiently. Actual spin speed in RPM will depend on rotor size
	Wash Solution is too concentrated.	Confirm that the Wash Solution Concentrate was diluted with the specified volume of ethanol. Keep the bottle tightly capped between uses to prevent evaporation
	Culture is too old.	Streak a fresh plate from a freezer stock, pick a single colony, and prepare a new culture.
	Too many or too few cells were used.	Confirm the cell density by measuring OD600. To calculate the volume of culture to use, take the desired cell mass (300 for high copy plasmids or 500 for low copy plasmids) and divide by the absorbance of the overnight culture at 600 nm.
	Plasmid replication is poor.	Confirm that the cells were grown in an appropriate medium under optimized conditions.
	Antibiotic activity is insufficient.	Use a fresh antibiotic solution for growth of overnight cultures. Most antibiotic solutions are light sensitive and degrade during long-term storage at 2–8 °C.
	Alkaline lysis is prolonged.	Reduce the lysis time (step 3) to 3 minutes or until the suspended cells form a clear viscous solution.
	Precipitation of cell debris is incomplete.	Reduce the initial volume of cell culture.
	Lysis is incomplete.	Reduce the initial volume of culture or increase the lysis time (step 3) while monitoring the lysis visually. For best results, use a total cell mass of 300 for high copy plasmids or 500 for low copy plasmids.
Absorbance of purified DNA does not accurately reflect quantity of the plasmid (A260/A280 ratio is high or low).	Wash Solution is diluted with ethanol containing impurities.	Check the absorbance of the ethanol between 250 and 300 nm. Do not use ethanol with high absorbance. Traces of impurities may remain on the binding column after washing and contribute to the absorbance in the final product.
	Plasmid DNA is contaminated with RNA; RNase A treatment is insufficient.	Confirm that the RNase A Solution was added to the Resuspension Solution prior to first use. The RNase A Solution may degrade due to high temperatures (>65 °C) or prolonged storage (>6 months at room temperature).
	Plasmid DNA is contaminated with chromosomal DNA.	Do not use cultures that have grown for more than 24 hours or are in the cell death phase. Do not vortex or vigorously shake the cells during the lysis reaction (step 3) or neutralization procedure (step 4).
	Background reading is high due to silica fines.	Spin the DNA sample at maximum speed for 1 minute; use the supernatant to repeat the absorbance readings.
	Purification is incomplete due to column overloading.	Reduce the initial volume of culture.

Troubleshooting Guide (continued)

Additional band is migrating ahead of the supercoiled plasmid during electrophoresis.	A portion of the plasmid DNA is permanently denatured.	Do not allow the lysis reaction (step 3) to exceed 5 minutes. Note that the nicked (covalently open) double-stranded plasmid DNA runs slower than the supercoiled DNA during electrophoresis.
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, and then resuspend the DNA in a smaller volume of water or Elution Solution. Alternatively, 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 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. Take up in water or Elution Solution. Note that the Elution Solution contains EDTA, which may chelate divalent cations (e.g. Mg^{2+}) that 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 washing (step 8) to remove any residual Wash Solution.

Related Products

- Water, Molecular Biology Reagent, Product Code W4502
- LB Broth, EZMix™, Product Code L7658
- LB Agar, EZMix, Product Code L7533
- Terrific Broth, EZMix, Product Code T9179
- Precast Agarose Gels, 1.0%, 8-well, Product Code P5472
- TAE Buffer (10X), Product Code T9650
- TBE Buffer (10X), Product Code T4415
- Gel Loading Solution, Product Code G2526
- DirectLoad™ Wide Range DNA Marker, Product Code D7058
- Ethidium bromide, aqueous, 10 mg/ml, Product Code E1510
- GenElute Plasmid Midiprep Kits, Product Code PLD35 and PLD140
- GenElute Plasmid Miniprep Kits, Product Code PLN10, PLN70, and PLN350

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

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