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Product Information

GenElute™ HP Plasmid Miniprep Kit

Catalog Numbers **NA0150S**, **NA0150**, and **NA0160**

TECHNICAL BULLETIN

Product Description

The GenElute™ HP Plasmid Miniprep 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 or vacuum column format, up to 25 µg of high copy plasmid DNA can be recovered from 1-5 ml of *E. coli* culture in less than 30 minutes. Note that actual yield and optimum volume of culture to use depend on the plasmid and the culture medium (see Procedure, step 1 on page 2).

An overnight recombinant *E. coli* culture is harvested with centrifugation and subjected to a modified alkaline-SDS lysis procedure followed by adsorption of the plasmid DNA onto silica in the presence of high

salts.^{1,2} Contaminants are then removed by a vacuum or spin wash step. Finally, the bound plasmid 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	Catalog Number	NA0150S 10 Preps	NA0150 70 Preps	NA0160 350 Preps
Resuspension Solution	R1149	2.5 ml	15.5 ml	100 ml
RNase A Solution	R6148	0.25 ml	0.25 ml	0.6 ml
Lysis Buffer	L1912	2.5 ml	15.5 ml	100 ml
Neutralization/Binding Buffer	N5158	4 ml	65 ml	140 ml
Column Preparation Solution	C2112	7 ml	60 ml	225 ml
Wash Solution 1	W0263	7 ml	50 ml	225 ml
Wash Solution 2	W4639	2.5 ml	12 ml	75 ml
Elution Solution (10 mM Tris-HCl, pH 8.5)	E7777	1.5 ml	8 ml	45 ml
GenElute HP Miniprep Binding Columns	G8667	10 each	70 each	5 x 70 each
Collection Tubes, 2.0 ml capacity	T5449	2 x 10 each	2 x 70 each	10 x 70 each

Equipment and reagents required but not provided

- Ethanol (95-100%), Catalog Numbers E7148, E7023, or 459836
- Microcentrifuge
- Microcentrifuge tubes
- Vacuum manifold with Luer fittings

Storage

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

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

The Neutralization/Binding Buffer and Wash Solution 1 contain guanidine. The Column Preparation Solution is caustic. Avoid contact with skin. Wear gloves, safety glasses, and suitable protective clothing when handling these solutions or other reagents.

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 and allow to cool to room temperature before use.
2. **Resuspension Solution:** Spin the tube of the RNase A Solution (Catalog Number R6148) briefly to collect the solution in the bottom of the tube. Add 13 µl for 10 prep package, 78 µl for 70 prep package, or 500 µl for 350 prep package, of the RNase A Solution to the Resuspension Solution prior to initial use. Note on the label that RNase A was added. Store at 2-8 °C.
3. **Wash Solution:** Dilute Wash Solution 2 with 95-100% ethanol prior to initial use: add 10 ml for the 10 prep package, 48 ml for the 70 prep package, or 300 ml for the 350 prep package. After each use, tightly cap the diluted wash solution to prevent the evaporation of ethanol.

Procedure

Note: All steps are carried out at room temperature.

1. **Harvest cells.** Pellet 1-5 ml of an overnight recombinant *E. coli* culture by centrifugation. The optimum volume of culture to use depends upon the plasmid and culture density. For best yields, follow the instructions in the note below. Transfer the appropriate volume of the recombinant *E. coli* culture to a microcentrifuge tube and pellet cells at $\geq 12,000 \times g$ for 1 minute. Discard the supernatant.
Note: For maximum plasmid recovery, begin with a single colony from a freshly streaked plate. Grow in medium containing the appropriate antibiotic at 37 °C with vigorous shaking (250-300 rpm) overnight. For best results with recombinant *E. coli* grown in LB (Luria Broth), use 1-3 ml of culture for high copy plasmids or 1-5 ml of culture for low copy plasmids. With recombinant *E. coli* grown in rich media such as TB (Terrific Broth) or 2X YT, use only 1 ml of culture. Higher volumes can cause a reduction in yield.
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 200 µl of the Resuspension Solution containing RNase A. Vortex or pipet up and down to thoroughly resuspend the cells until homogeneous. Incomplete resuspension will result in poor recovery.

Another rapid way to resuspend the cell pellets is to scrape the bottoms of the microcentrifuge tubes back and forth 5 times across the surface of a polypropylene microcentrifuge tubes storage rack with 5 x 16 holes.³

3. **Cell Lysis .** Lyse the resuspended cells by adding 200 µl 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 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 and render it unsuitable for most applications.
4. **Neutralization** Precipitate the cell debris by adding 350 µl of the Neutralization/Binding Buffer. Gently invert the tube 4-6 times. Pellet the cell debris by centrifuging at $\geq 12,000 \times g$ or maximum speed for 10 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 particulates after centrifugation, recentrifuge the supernatant before proceeding to step 6.

A. Vacuum Method

Note: Use the spin format for better results with EndA⁺ strains of *E. Coli*.

5. **Prepare Column.** During the centrifugation in step 4, set up the vacuum manifold and insert a GenElute HP Miniprep Binding Column. Add 500 µl of the Column Preparation Solution to each miniprep column and apply the vacuum until the entire solution passes through the column. Switch off vacuum source.
Note: The Column Preparation Solution maximizes binding of DNA to the membrane resulting in more consistent yields.
6. **Load cleared lysate.** Transfer the cleared lysate from step 4 to the column and apply the vacuum until the entire lysate passes through the column. Switch off vacuum source.
7. **Wash column with Wash Solution 1.** Add 500 µl of the Wash Solution 1 to the column. Apply the vacuum until the entire solution has passed through the column. Switch off vacuum source.
Note: Use of Wash Solution 1 lowers levels of some contaminants, such as endotoxins.

8. **Wash column with Wash Solution 2.** Prior to first time use, be sure to add ethanol to Wash Solution 2. Add 750 μl of the diluted Wash Solution 2 to the column. And apply the vacuum until the entire solution passes through the column. Switch off vacuum source
Note: Wash Solution 2 removes residual salt and other contaminants introduced during the column load
9. Transfer the column to a provided 2 ml microcentrifuge tube and centrifuge at $\geq 12,000 \times g$ for 1 minute to remove excess ethanol.
10. **Elute DNA.** Transfer the column to a fresh collection tube. Add 100 μl of Elution Solution (10 mM Tris-HCl, pH 8.5) or molecular biology reagent water (not included) to the column. Centrifuge at $\geq 12,000 \times g$ for 1 minute. 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 50 μl . However, this may result in a reduction in the total plasmid DNA yield.

B. Spin Method

5. **Prepare Column.** Insert a GenElute HP Miniprep Binding Column into a provided microcentrifuge tube, if not already assembled. Add 500 μl of the Column Preparation Solution to each miniprep column and centrifuge at $\geq 12,000 \times g$ for 30 seconds to 1 minute. Discard flow-through liquid.
Note: The Column Preparation Solution maximizes binding of DNA to the membrane resulting in more consistent yields.
6. **Load cleared lysate.** Transfer the cleared lysate from step 4 to the column and centrifuge at $\geq 12,000 \times g$ for 30 seconds to 1 minute. Discard the flow-through liquid.
7. **Wash column with Wash Solution 1.** Add 500 μl of the Wash Solution 1 to the column. Centrifuge at $\geq 12,000 \times g$ for 30 seconds to 1 minute. Discard the flow-through liquid.
Note: Use of Wash Solution 1 lowers levels of some contaminants, such as endotoxins.
8. **Wash column with Wash Solution 2.** Prior to first time use, be sure to add ethanol to Wash Solution 2. Add 750 μl of the diluted Wash solution 2 to the column. Centrifuge at $\geq 12,000 \times g$ for 30 seconds to 1 minute. Discard the flow-through liquid.
Note: Wash Solution 2 removes residual salt and other contaminants introduced during the column load.

9. Centrifuge at $\geq 12,000 \times g$ for 1 minutes to remove excess ethanol.
10. **Elute DNA.** Transfer the column to a fresh collection tube. Add 100 μl of Elution Solution (10 mM Tris-HCl, pH 8.5) or molecular biology reagent water (not included) to the column. Centrifuge at $\geq 12,000 \times g$ for 1 minute. 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 50 μl . However, this may result in a reduction in the total plasmid DNA yield.

Results

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

References

1. Birnboim, H.C., and Doly, J., A rapid alkaline extraction procedure for screening recombinant plasmid DNA. *Nucleic Acids Res.*, **7**, 1513-1522 (1979).
2. Vogelstein, B., and Gillespie, D., Preparative and analytical purification of DNA from agarose. *Proc. Natl. Acad. Sci. USA*, **76**, 615-619 (1979).
3. Voo, K. S., and Jacobsen, B. M., Rapid resuspension of pelleted bacterial cells for miniprep plasmid DNA isolation. *BioTechniques*, **24**, 240-243 (1998).

Related Products

- LB Broth, Catalog Number L2542
- TAE Buffer (10 \times), Catalog Number T9650
- TBE Buffer (10 \times), Catalog Number T4415
- Gel Loading Buffer, Catalog Number G2526
- DirectLoad™ Wide Range DNA Marker, Catalog Number D7058
- Ethidium bromide, aqueous, 10 mg/ml, Catalog Number E1510
- GenElute HP Plasmid Maxiprep Kits, Catalog Numbers NA0300S, NA0300, NA0310
- GenElute HP Plasmid Midiprep Kits, Catalog Numbers NA0200S, NA0200
- GenElute HP Endotoxin-Free Maxiprep Kits, Catalog Numbers NA0400S, NA0400, NA0410

Troubleshooting Guide

Problem	Cause	Solution
Poor or low plasmid DNA recovery	Wash Solution 2 is too concentrated.	Confirm that the specified volume of ethanol was added. Keep the bottle tightly capped between uses to prevent evaporation.
	Number of cells is insufficient.	Culture may be too old. Prepare a new culture. Or Confirm cell density. Grow culture to OD ₆₀₀ = 2.0-3.0.
	Started with too much culture.	Use less culture next time. Do not use more than 5 ml of culture from LB or 1 ml of culture from TB or other rich medium. Exceeding the recommended volumes can reduce yields.
	Plasmid replication is poor.	Use only cells grown in suitable media under optimal 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 clear, viscous solutions.
	Precipitation of cell debris is incomplete.	Reduce the initial volume of cell culture.
Absorbance of purified DNA does not accurately reflect quantity of plasmid (A ₂₆₀ /A ₂₈₀ ratio is high or low).	Lysis is incomplete.	Reduce the initial volume of cell culture or increase the lysis time (step 3) while monitoring the lysis visually.
	Purification is incomplete due to column overloading.	Reduce the initial volume of culture.
	Background reading is high due to silica fines.	Spin DNA sample at maximum speed for 1 minute, use supernatant to repeat absorbance readings.
	Wash Solution 2 is diluted with ethanol containing impurities.	Check absorbance of the ethanol at 250-300 nm. Do not use if absorbance is high. Traces impurities remaining on the binding column after washing may add to absorbance of final product.
	DNA is contaminated with RNA; RNase A treatment is insufficient.	Confirm that RNase A was added to the Resuspension Solution before use. The RNase A Solution may degrade due to high temperatures (>65 °C) or prolonged storage (>6 months at RT)
Additional band migrating ahead of supercoiled plasmid.	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 or neutralization procedure.
	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 solution at 65 °C until dissolved. Cool to RT before use.
	DNA concentration is too low.	Precipitate the DNA with ethanol, then resuspend the DNA in a smaller volume of Elution Solution or water. 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 Wash Step with Wash Solution 1 (step 7) must be included when recovering DNA from EndA ⁺ strains and use spin format only.
	Final plasmid DNA eluate contains too much salt.	Precipitate the DNA using ethanol. Dry the pellet. Redissolve in water or Elution Solution.
	Column contains residual Wash Solution 2	Re-centrifuge the column for 1 minute after washing (step 8) to remove any residual Wash Solution.

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