User Guide

Catalog Nos.
NA0150S
NA0150
NA0160

GenElute™ HP Plasmid Miniprep Kit

sigma.com

Ordering Information

Cat. No.	Product Description	Pkg Size
NA0150S	GenElute HP Plasmid Miniprep Kit	10 preps
NA0150	GenElute HP Plasmid Miniprep Kit	70 preps
NA0160	GenElute HP Plasmid Miniprep Kit	350 preps

Related Products

Cat. No.	Product Description	Pkg Size
L2542	LB Broth	500 mL
T9650	TAE Buffer (105)	1 L
T4415	TBE Buffer (105)	1 L
G2526	Gel Loading Solution	5 mL
D7058	DirectLoad™ Wide Range DNA Marker	1 vl
E1510	Ethidium bromide, aqueous, 10mg/mL	10 mL
NA0300S	GenElute HP Plasmid Maxiprep Kit	4 preps
NA0300	GenElute HP Plasmid Maxiprep Kit	10 preps
NA0310	GenElute HP Plasmid Maxiprep Kit	25 preps
NA0200S	GenElute HP Plasmid Midiprep Kit	4 preps
NA0200	GenElute HP Plasmid Midiprep Kit	25 preps
NA0400S	GenElute HP Endotoxin-Free Plasmid Maxiprep Kit	4 preps
NA0400	GenElute HP Endotoxin-Free Plasmid Maxiprep Kit	10 preps
NA0410	GenElute HP Endotoxin-Free Plasmid Maxiprep Kit	25 preps

To reorder product call 1-800-325-3010, visit our Web site at sigma-aldrich.com, or contact your local sales representative.

GenElute HP Plasmid Miniprep Kit

Table of Contents

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 No.	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 or T7813**	2 x 10 each	2 x 70 each	10 x 70 each

Equipment and Reagents Required But Not Provided

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

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.

Storage and Stability

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

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 (**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 ≥12,000 x *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

Important Reminder: *Verify that the appropriate volume of RNase A solution was added to the Resuspension Solution.*

Completely resuspend the bacterial pellet with 200 µl of the Resuspension Solution containing RNase A. Vortex or pipette 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 ≥12,000 x *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**

Important Reminder: *Verify that ethanol has been added to the bottle of Wash Solution 2.*

Add 750 µL of the diluted Wash Solution 2 to the column. 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**

Transfer the column to a provided 2 mL microcentrifuge tube and centrifuge at ≥12,000 x *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 ≥12,000 x *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 ≥12,000 x *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 ≥12,000 x *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 ≥12,000 x *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

Important Reminder: *Verify that ethanol has been added to the bottle of Wash Solution 2.*

Add 750 µL of the diluted Wash Solution 2 to the column. Centrifuge at ≥12,000 x *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	Centrifuge at ≥12,000 x *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 ≥12,000 x *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.; Doly, J. A rapid alkaline extraction procedure for screening recombinant plasmid DNA. *Nucleic Acids Res.* **1979**, *7*, 1513.
2. Vogelstein, B.; Gillespie, D. Preparative and analytical purification of DNA from agarose. *Proc. Natl. Acad. Sci. U.S.A.* **1979**, *76*, 615.
3. Voo, K. S.; Jacobsen, B. M. Rapid resuspension of pelleted bacterial cells for miniprep plasmid DNA isolation. *BioTechniques* **1998**, *24*, 240.

Troubleshooting Guide

Poor or low plasmid DNA recovery	**Cause** — Wash Solution 2 is too concentrated. **Solution** — Confirm that the specified volume of ethanol was added. Keep the bottle tightly capped between uses to prevent evaporation.
	Cause — Number of cells is insufficient. **Solution** — Culture may be too old. Prepare a new culture. *OR* Confirm cell density. Grow culture to OD600 = 2.0–3.0.
	Cause — Started with too much culture. **Solution** — 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.
	Cause — Plasmid replication is poor. **Solution** — Use only cells grown in suitable media under optimal conditions.
	Cause — Antibiotic activity is insufficient. **Solution** — 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.
	Cause — Alkaline lysis is prolonged. **Solution** — Reduce the lysis time (step 3) to 3 minutes or until the suspended cells form clear, viscous solutions.
	Cause — Precipitation of cell debris is incomplete. **Solution** — Reduce the initial volume of cell culture.
	Cause — Lysis is incomplete. **Solution** — Reduce the initial volume of cell culture or increase the lysis time (step 3) while monitoring the lysis visually.

Absorbance of purified DNA does not accurately reflect quantity of plasmid (A_{260}/A_{280} ratio is high or low).	**Cause** — Purification is incomplete due to column overloading. **Solution** — Reduce the initial volume of culture.
	Cause — Background reading is high due to silica fines. **Solution** — Spin DNA sample at maximum speed for 1 minute, use supernatant to repeat absorbance readings.
	Cause — Wash Solution 2 is diluted with ethanol containing impurities. **Solution** — Check absorbance of the ethanol at 250–300 nm. Do not use if absorbance is high. Trace impurities remaining on the binding column after washing may add to absorbance of final product.
	Cause — DNA is contaminated with RNA; RNase A treatment is insufficient. **Solution** — 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)
	Cause — Plasmid DNA is contaminated with chromosomal DNA. **Solution** — 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.
Additional band migrating ahead of supercoiled plasmid.	**Cause** — A portion of the plasmid DNA is permanently denatured. **Solution** — 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	**Cause** — Purification is incomplete. **Solution** — Salts in one or more of the solutions may have precipitated. Heat solution at 65 °C until dissolved. Cool to room temperature before use.
	Cause — DNA concentration is too low. **Solution** — 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.
	Cause — DNA was prepared from EndA$^+$ strains. **Solution** — The Wash Step with Wash Solution 1 (step B.7) must be included when recovering DNA from EndA$^+$ strains; use spin format only.
	Cause — Final plasmid DNA eluate contains too much salt. **Solution** — Precipitate the DNA using ethanol. Dry the pellet. Redissolve in water or Elution Solution.
	Cause — Column contains residual Wash Solution 2. **Solution** — Re-centrifuge the column for 1 minute after washing (step B.8) to remove any residual Wash Solution.

Notes

Experienced User Protocol: Vacuum Method

- Preparation:
  - Add RNase A to the Resuspension Solution in the following volumes:
    - 13 µL for a 10 prep package
    - 78 µL for a 70 prep package
    - 500 µL for a 350 prep package
  - Add 95–100% Ethanol to Wash Solution 2 in the following volumes:
    - 10 mL for a 10 prep package
    - 48 mL for a 70 prep package
    - 300 mL for a 350 prep package

1 Harvest & Lyse Bacteria

- Pellet 1–5 mL of an overnight culture at ≥12,000 x *g* for 1 minute. Discard supernatant.
- Resuspend cells in 200 µL of Resuspension Solution. Pipette up and down to mix.
- Add 200 µL of Lysis Buffer and gently invert 6–8 times to mix. Do not vortex. Allow to clear, 3–5 minutes.

2 Prepare Cleared Lysate

- Add 350 µL of Neutralization/Binding Buffer and gently invert 4–6 times to mix.
- Pellet cell debris at ≥12,000 x *g* for 10 minutes. If supernatant (cleared lysate) contains a large amount of floating precipitates, re-centrifuge before loading it into the binding column.

3 Prepare Column

- Place Miniprep Binding Column on vacuum manifold.
- Add 500 µL of Column Preparation Solution to Miniprep Binding Column.
- Apply vacuum until all solution passes through the column; switch off vacuum source.

4 Bind Plasmid DNA to Column

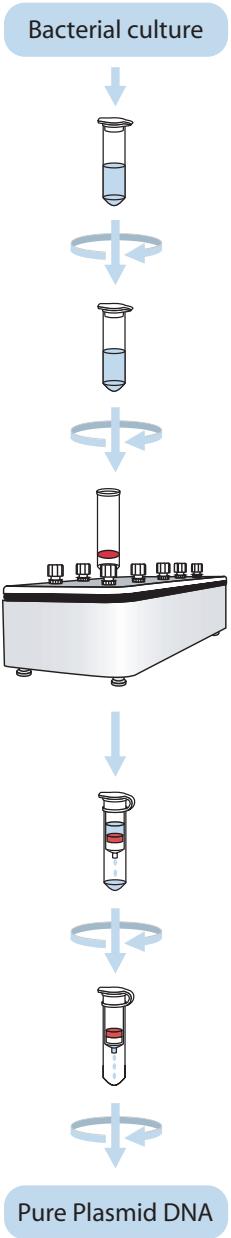
- Transfer the cleared lysate from step 2 to the column and apply vacuum until entire lysate passes through the column; switch off vacuum source.

5 Wash to Remove Contaminants

- Add 500 µL of Wash Solution 1 to the column and apply vacuum until all solution has passed through the column; switch off vacuum source.
- Add 750 µL of Wash Solution 2 to the Binding Column and apply vacuum until all solution has passed through the column; switch off vacuum source.
- Transfer the Binding Column to a 2 mL microcentrifuge tube and centrifuge at ≥12,000 x *g* for 1 minute to remove excess ethanol.

6 Elute Purified Plasmid DNA

- Transfer the Binding Column to a fresh collection tube.
- Add 100 µL of Elution Solution to the column.
- Centrifuge at ≥12,000 x *g* for 1 minute.
- For More Concentrated Plasmid DNA: Reduce addition of Elution Solution to 50 µL. Centrifuge at ≥12,000 x *g* for 1 minute.



Experienced User Protocol: Spin Method

- **Preparation**:
  - Add RNase A to the Resuspension Solution in the following volumes:
    - 13 µL for a 10 prep package
    - 78 µL for a 70 prep package
    - 500 µL for a 350 prep package
  - Add 95–100% Ethanol to Wash Solution 2 in the following volumes:
    - 10 mL for a 10 prep package
    - 48 mL for a 70 prep package
    - 300 mL for a 350 prep package

1 Harvest & Lyse Bacteria

- Pellet 1–5 mL of an overnight culture at ≥12,000 x *g* for 1 minute. Discard supernatant.
- Resuspend cells in 200 µL of Resuspension Solution. Pipette up and down to mix.
- Add 200 µL of Lysis Buffer and gently invert 6–8 times to mix. Do not vortex. Allow to clear, 3–5 minutes.

2 Prepare Cleared Lysate

- Add 350 µL of Neutralization/Binding Buffer and gently invert 4–6 times to mix.
- Pellet cell debris at ≥12,000 x *g* for 10 minutes. If supernatant (cleared lysate) contains a large amount of floating precipitates, re-centrifuge before loading it into the binding column.

3 Prepare Column

- Insert Miniprep Binding Column into provided microcentrifuge tube.
- Add 500 µL of Column Preparation Solution to Miniprep Binding Column.
- Centrifuge at ≥12,000 x *g* for 30 seconds to 1 minute. Discard flow-through liquid.

4 Bind Plasmid DNA to Column

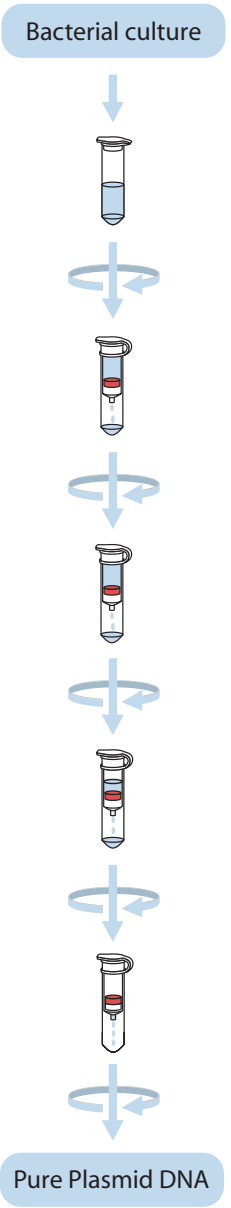
- Transfer the cleared lysate from step 2 to the column and centrifuge at ≥12,000 3 *g* for 30 seconds to 1 minute. Discard flow-through liquid.

5 Wash to Remove Contaminants

- Add 500 µL of Wash Solution 1 to the column and centrifuge at ≥12,000 x *g* for 30 seconds to 1 minute. Discard flow-through liquid.
- Add 750 µL of Wash Solution 2 to the Binding Column and centrifuge at ≥12,000 x *g* for 30 seconds to 1 minute. Discard flow-through liquid.
- Centrifuge at ≥12,000 x *g* for an additional 1 minute to remove excess ethanol.

6 Elute Purified Plasmid DNA

- Transfer the Binding Column to a fresh collection tube.
- Add 100 µL of Elution Solution to the column.
- Centrifuge at ≥12,000 3 *g* for 1 minute.
- For More Concentrated Plasmid DNA: Reduce addition of Elution Solution to 50 µL. Centrifuge at ≥12,000 3 *g* for 1 minute.



Enabling Science to
Improve the Quality of Life

Order/Customer Service: sigma-aldrich.com/order
Technical Service: sigma-aldrich.com/techservice
Development/Custom Manufacturing Inquiries
SAFC® safcglobal@sial.com
Safety-related Information: sigma-aldrich.com/safetycenter

World Headquarters
3050 Spruce St.
St. Louis, MO 63103
(314) 771-5765
sigma-aldrich.com

The PCR process is covered by patents owned by Hoffman-LaRoche, Inc.
PicoGreen is a registered trademark of Molecular Probes, Inc.

0026-502620
1094