User Guide

Catalog Nos.

PLN10

PLN70

PLN350

GenElute™ Plasmid Miniprep Kit

sigma.com

Ordering Information

Catalog No.	Product Description	Pkg Size
PLN10	GenElute Plasmid Miniprep Kit	10 preps
PLN70	GenElute Plasmid Miniprep Kit	70 preps
PLN350	GenElute Plasmid Miniprep Kit	350 preps

Related Products

Catalog No.	Product Description	Pkg Size
PFM10	GenElute Five-Minute Miniprep Kit	10 preps
PFM50	GenElute Five-Minute Miniprep Kit	50 preps
PFM250	GenElute Five-Minute Miniprep Kit	250 preps
NA0200S	GenElute HP Plasmid Midiprep Kit	4 preps
NA0200	GenElute HP Plasmid Midiprep Kit	25 preps
NA0300S	GenElute HP Plasmid Maxiprep Kit	4 preps
NA0300	GenElute HP Plasmid Maxiprep Kit	10 preps
NA0310	GenElute HP Plasmid Maxiprep Kit	25 preps
NA0400	GenElute HP Endotoxin-Free Plasmid Maxiprep Kit	4 preps
NA0410	GenElute HP Endotoxin-Free Plasmid Maxiprep Kit	10 preps
NA0500	GenElute HP Plasmid Megaprep Kit	5 preps
NA0600	GenElute HP Endotoxin-Free Plasmid Megaprep Kit	5 preps

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GenElute™ Plasmid Miniprep Kit

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Product Description

The GenElute 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 column format, up to 15 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 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 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 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	Catalog No.	PLN10 10 Preps	PLN70 70 Preps	PLN350 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 Solution	**L1912**	2.5 ml	15.5 ml	100 ml
Neutralization/Binding Solution	**N5158**	4 ml	65 ml	140 ml
Column Preparation Solution	**C2112**	7 ml	60 ml	225 ml
Optional Wash Solution	**W4011**	5.5 ml	40 ml	200 ml
Wash Solution Concentrate	**W3886**	2.5 ml	25 ml	75 ml
Elution Solution (10 mM Tris-HCl, 1 mM EDTA, pH approx. 8.0)	**E5650**	1.5 ml	8 ml	45 ml
GenElute Miniprep Binding Columns	**G6415**	10 each	70 each	350 each
2 ml Collection Tubes	**T5449 or T7813**	20 each	140 each	700 each

Equipment and Reagents Required But Not Provided

- Ethanol (95–100%), Catalog Nos. **E7148**, **E7023**, or **459836**
- Microcentrifuge
- Microcentrifuge tubes

Precautions and Disclaimer

The GenElute Plasmid Miniprep Kit 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.

Storage and Stability

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

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 No. **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. Store at 4 °C.

3. **Wash Solution**

   Dilute the Wash Solution Concentrate with 10 ml (10 prep package), 100 ml (70 prep package), or 300 ml (350 prep package) of 95–100% ethanol prior to initial use. After each use, tightly cap the diluted wash solution to prevent the evaporation of ethanol.

Procedure

Note: All centrifugation speeds are given in units of *g*. Please refer to Table 1 for information on converting g-force to rpm. If centrifuges/rotors for the required *g*-forces are not available, use the maximum *g*-force possible and increase the spin time proportionally. Spin until all liquid passes through the column.

All steps are carried out at room temperature.

Harvest cells

Pellet 1–5 ml of an overnight recombinant *E. coli* culture by centrifugation. The optimal 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 3 *g* for 1 minute. Discard the supernatant.

Note: 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.

1. Resuspend cells

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

Completely resuspend the bacterial pellet with 200 µl of the Resuspension Solution. 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 tube storage rack with 5 x 16 holes.[3]

2. Lyse cells

Lyse the resuspended cells by adding 200 µl of the Lysis Solution. 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 that may render it unsuitable for most downstream applications.

3. Neutralize

Precipitate the cell debris by adding 350 µl of the Neutralization/Binding Solution. 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.

4. **Prepare Column**

Insert a GenElute 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 the flow-through liquid.

Note: The Column Preparation Solution maximizes binding of DNA to the membrane resulting in more consistent yields.

5. **Load cleared lysate**

Transfer the cleared lysate from step 3 to the column prepared in step 4 and centrifuge at ≥12,000 x *g* for 30 seconds to 1 minute. Discard the flow-through liquid.

6. **Optional wash (use only with EndA$^+$ strains)**

Add 500 µl of the Optional Wash Solution to the column. Centrifuge at ≥12,000 x *g* for 30 seconds to 1 minute. Discard the flow-through liquid.

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. **Wash column**

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

Add 750 µl of the diluted Wash Solution to the column. Centrifuge at ≥12,000 x *g* for 30 seconds to 1 minute. The column wash step removes residual salt and other contaminants introduced during the column load. Discard the flow-through liquid and centrifuge again at maximum speed for 1 to 2 minutes without any additional Wash Solution to remove excess ethanol.

8. **Elute DNA**

Transfer the column to a fresh collection tube. Add 100 µl 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 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. The 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. BioTechiques, **24**, 240-243 (1998).

Troubleshooting Guide

Poor or no plasmid DNA recovery	**Cause** — Wash Solution is too concentrated. **Solution** — Confirm that the Wash Solution Concentrate was diluted with the specified volume of ethanol. 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 OD_{600} = 2.0–3.0.
	Cause — Plasmid replication is poor. **Solution** — Confirm that cells were grown in an appropriate medium under optimized 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 a clear, viscous solution.
	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 is diluted with ethanol containing impurities. **Solution** — 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.
	Cause — DNA is contaminated with RNA; RNase A treatment is insufficient. **Solution** — 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).
	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 (step 3) or neutralization procedure (step 4).
Additional band is migrating ahead of supercoiled plasmid during electrophoresis.	**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 the solution at 65 °C until dissolved. Cool to room temperature prior to 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 Optional Wash Step (step 7) must be included when recovering DNA from EndA$^+$ strains.
	Cause — The final plasmid DNA eluate contains too much salt. **Solution** — Precipitate the DNA using ethanol. Dry the pellet. Redissolve in water or Elution Solution. Note that the Elution Solution contains EDTA, which may chelate divalent cations (e.g., Mg^{2+}) which are important co-factors for many enzymes.
	Cause — The column contains residual ethanol from the diluted Wash Solution. **Solution** — Re-centrifuge the column for 1 minute after washing (step 8) to remove any residual Wash Solution.

Related Products	Catalog No.	Related Products	Catalog No.
Water, Molecular Biology Reagent	W4502	Ethidium bromide, aqueous, 10 mg/ml	E1510
Precast Agarose Gels, 1.0%, 8 well	P5472	GenElute HP Plasmid Maxiprep Kits	NA0300S NA0300 NA0310
TAE Buffer (10X)	T9650	GenElute HP Plasmid Midiprep Kits	NA0200S NA0200
Gel Loading Solution	G2526	TBE Buffer (10X)	T4415
DirectLoad™ Wide Range DNA Marker	D7058		

Appendix

Table 1. Conversion of Centrifugal Force (in units of g) to RPM for Common Rotors

Centrifuge	Rotor	Tubes (max)	Radius (cm)	RPM at 300 x *g*	RPM at 12,000 x *g*	RPM at 16,000 x *g*
Eppendorf 5410		12	5.8	2,143	13,555	15,652
5415C	F45-18-11	18	7.3	1,917	12,124	14,000
5415D&R	F45-24-11	24	8.3	1,801	11,392	13,155
5417C,D,&R	F45-30-11	30	9.5	1,681	10,634	12,279

See table above for spin speeds in rpm for selected common centrifuges and rotors. The correct rpm for unlisted rotors can be calculated using the formula:

$$RPM = \sqrt{RCF} / 1.118 \times 10^{-5} r$$

where *RCF* = required gravitational acceleration (relative centrifugal force) in units of *g*;
r = radius of the rotor in cm;
RPM = the number of revolutions per minute required to achieve the necessary *g*-force

Notes

Experienced User Protocol

All spins at ;12,000 3 g, except as noted.

1 Harvest & lyse bacteria

- Pellet cells from 1–5 ml overnight culture *1 minute* (1 ml from TB or 2xYT; 1–5 ml from LB medium). Discard supernatant.
- Resuspend cells in 200 µl Resuspension Solution. Pipette up and down or vortex.
- Add 200 µl of Lysis Solution. Invert gently to mix. Do not vortex. Allow to clear for :5 minutes

* *Prior to first time use, be sure to add the RNase A to the Resuspension Solution.*

2 Prepare cleared lysate

- Add 350 µl of Neutralization Solution (S3). Invert 4–6 times to mix.
- *Pellet debris 10 minutes at max speed.*

3 Prepare binding column

- Add 500 µl Column Preparation Solution to binding column in a collection tube.
- *Spin at ≥12,000 x g, 1 minute.* Discard flow-through.

4 Bind plasmid DNA to column

- Transfer cleared lysate into binding column.
- *Spin 30",1 minute.* Discard flow-through.

5 Wash to remove contaminants

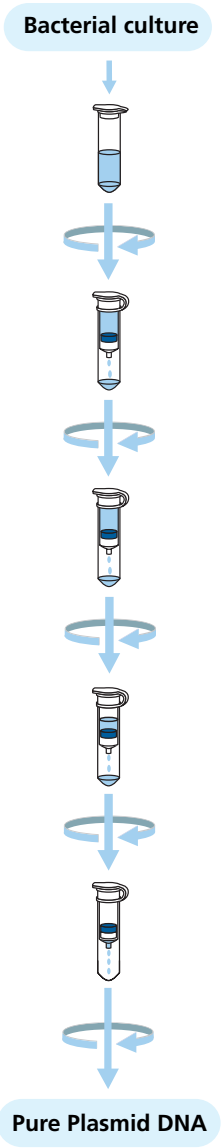
- *Optional (EndA$^+$ strains only):* Add 500 µl Optional Wash Solution to column. *Spin 30", 1 minute.* Discard flow-through.
- Add 750 µl Wash Solution to column. *Spin 30", 1 minute.* Discard flow-through.
- *Spin 1 minute* to dry column.

* *Prior to first time use, be sure to add ethanol to the concentrated Wash Solution.*

6 Elute purified plasmid DNA

- Transfer column to new collection tube.
- Add 100 µl Elution Solution. *Spin 1 minute.*

* *If a more concentrated plasmid DNA prep is required, reduce the elution volume to a minimum of 50 µl.*



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