User Guide

Catalog Nos.
NA0600

GenElute™ HP Endotoxin-Free Plasmid Megaprep Kit

sigma.com

Ordering Information

Cat. No.	Product Description	Pkg Size
NA0600	GenElute HP Endotoxin-Free Plasmid Megaprep Kit	5 preps

Related Products

Cat. No.	Product Description	Pkg Size
NA0150	GenElute HP Plasmid Miniprep Kit	70 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
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
NA0500	GenElute HP Plasmid Megaprep Kit	5 preps
NA0800	GenElute HP Select Plasmid Gigaprep Kit	5 preps

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GenElute HP Endotoxin-Free Plasmid Megaprep Kit

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Product Description

The GenElute™ HP Endotoxin-Free Plasmid Megaprep Kit offers a simple, rapid, and cost-effective method for isolating endotoxin-free plasmid DNA from recombinant *E. coli* cultures. Up to 5 mg of high copy plasmid DNA with < 0.1 endotoxin unit/µg DNA can be recovered from *E. coli* cultures in 200 ml to 1 liter LB medium or 200–600 ml TB medium in less than 1.5 hours. Actual yield depends on the strain, the plasmid, and the culture medium used.

An overnight recombinant *E. coli* culture is harvested with centrifugation and subjected to a modified alkaline-SDS lysis. The lysate is cleared by filtration followed by the addition of a binding solution that has been optimized for endotoxin-free plasmid preparations. The plasmid DNA is then captured onto a silica membrane in the presence of high salts,[1,2] while endotoxins are prevented from adsorbing to the membrane. Contaminants are removed by two wash steps. Finally, the bound DNA is eluted in endotoxin-free water.

The recovered plasmid DNA is predominately in its supercoiled form. Genomic DNA and RNA are below detectable levels by ethidium bromide stained agarose gel electrophoresis. The DNA is ready for immediate use in downstream applications such as restriction digestion, ligation, sequencing, PCR, transformation, and transfection.

The GenElute HP Endotoxin-Free Plasmid Megaprep Kit delivers significant time-savings, overall higher yields and better transfection efficiencies compared to anion-exchange and other methods.

Reagents Provided	Cat. No.	Quantity
Resuspension Solution	**R1149**	240 ml
RNase A Solution	**R6148**	1.5 ml
Lysis Solution	**L1912**	240 ml
Lysate Clearing Agent	**L4667**	5 pks
Neutralization Solution	**N7411**	240 ml
Binding Solution	**B9560***	280 ml
Column Preparation Solution	**C2112**	2 x 225 ml
Wash Solution 1	**W0263**	225 ml
Wash Solution 2	**W4639**	75 ml
Endotoxin-Free Water	**2107**	120 ml
GenElute HP Megaprep Filters	**G6169**	5 each
GenElute HP Pro Megaprep Binding Columns	**G3546****	5 each
VacCaps	**R4778**	5 each
Collection Tubes, 50 ml capacity	**C4353**	5 each

*Sigma Life Sciences continually seeks ways to improve our products. Please note that the product code for Binding Solution has changed from B1810 to B9560. This change has been made to reflect an improvement to the manufacturing process. The new manufacturing process results in the same functionality and specifications of Binding Solution.

**Sigma Life Sciences continually seeks ways to improve our products. The design of the GenElute HP Megaprep Binding Columns (Product code G6294) has been improved. This improved product, GenElute HP Pro Megaprep Binding Columns (Product code G3546), will replace G6294. The performance and functionality of G3546 and G6294 are equivalent.

Equipment and Reagents Required But Not Provided

- Centrifuge capable of 5000 x *g*
- Vacuum manifold, Catalog No. **VM20**
- Vacuum source capable of ≥500 mbar (refer to Appendix 2 for unit conversions)
- Ethanol (95–100%), Catalog No. **E7148**, **E7023**, or **459836**
- Collection bottles (≥250 ml) with 45 mm neck, Catalog No. **CLS61626250**

Precautions and Disclaimer

The GenElute HP Endotoxin-Free Plasmid Megaprep 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. After the RNase A Solution is added to the Resuspension Solution, store at 2–8 °C. The Neutralization Solution can also be stored at 2–8 °C, since it is recommended to use this solution chilled in the protocol.

Preparation Instructions

1. Prepare a starter culture

Pick a single colony from a freshly streaked plate and inoculate a starter culture of 3-5 ml LB or TB medium. Use the appropriate antibiotic and incubate at 37 °C for approximately 8 hours while shaking at 250-300 rpm. Dilute the starter culture 1:500 to 1:1000 in the appropriate medium and incubate at 37 °C for 12-16 hours while shaking at 250-300 rpm.

A healthy culture generally reaches an absorbance at 600 nm between 2 and 4 in LB media and between 3 and 6 in TB media.

Note: For some low copy constructs, growing the culture in LB medium gives higher yield and lower endotoxin in final plasmid preparation than growth in TB medium.

2. Choosing the correct culture volume

Use of 200 ml to 1 liter of LB medium or 200-600 ml in TB medium generally results in good plasmid yields and endotoxin levels. However, the optimal volume of culture to use depends upon the strain, the plasmid, and the density of the culture since the number of bacterial cells can vary greatly between cultures.

Too few cells (low cell mass) will result in low DNA yields. Conversely, with too many cells (high cell mass) the bacteria may not lyse efficiently and cause poor release of the plasmid DNA or the potential to trap lysate volume in the cell debris during filtration resulting in a lower yield. By following the cell mass calculation, you will ensure maximum plasmid recovery from the overnight culture.

For best results, we recommend using a volume of culture based on cell mass. A total cell mass of 500–2500 for LB culture or 500–1500 for TB culture is recommended, but a **cell mass of 1500 in LB is typically optimal**. The optimal volume of culture to use can be calculated using the formula below:

$$\text{Volume}_{\text{optimal}} = \frac{1500}{A_{600}}$$

Pellet wet weight is another good factor to determine the amount of culture to use. Use of a pellet wet weight of 2–8 *g* per preparation is recommended.

3. Mix reagents thoroughly

Examine the reagents for precipitation. If a precipitate forms during storage, warm the reagent at 55–65 °C until the precipitate dissolves. Cool the reagent to room temperature before use.

4. Prepare Resuspension/ RNase A Solution

Spin the tube of RNase A Solution (**R6148**) briefly to collect the solution in the bottom of the tube. Add **1.25 ml of RNase A Solution** to the bottle of Resuspension Solution (**R1149**) prior to initial use. Store at 2–8 °C.

5. Prepare Wash Solution 2

Add 300 ml of 95–100% ethanol to the bottle of Wash Solution 2 (**W4639**) prior to initial use. After each use, tightly cap the diluted Wash Solution 2 to prevent the evaporation of ethanol.

6. Chill Neutralization Sollution

The Neutralization Solution (**N7411**) can be stored at 2–8 °C since it should be chilled prior to use.

Procedure

All steps are carried out at room temperature. When using vacuum, make certain the vacuum level is equal to or greater than 500 mbar (refer to *Appendix 2* for unit conversions). When handling more than one sample, use a 3-way stopcock or T tubing connector to split the vacuum connection.

Convenient stopping points

Step 1:

The wet bacterial pellet can be frozen at –70 °C for one month without any detrimental effects to the quality or yield of the plasmid DNA.

Step 2 and step 9:

Do not prepare Binding Column in Step 2. Instead perform Steps 1 and 3-9. Now cleared lysate containing Binding Solution can be stored overnight at 2-8 °C without any detrimental effects to the quality or yield of the plasmid DNA.

When you are ready to continue the plasmid purifications, prepare the Binding Column with Column Preparation Solution (C2112) as described in Step 2, **wait 10 minutes,** and continue with step 10. Follow the procedure to completion.

1. Harvest cells

Important Reminder: *The optimal volume of culture can be calculated based on cell mass. Refer to Preparation Instructions.*

Pellet **200 ml to 1 liter of LB or 200–600 ml in TB of an overnight culture** by centrifugation at 5000 x *g* for 10 minutes. Discard the supernatant.

2. Prepare binding column(s)

Attach the GenElute HP Pro Megaprep Binding Column(s) to a vacuum manifold. Prepare the binding column by adding **40 ml** of **Column Preparation Solution** to the Binding Column. Apply vacuum to draw all the liquid through the column, then turn the vacuum off.

3. Resuspend cells

Important Reminder: *Verify that 1.25 ml RNase A Solution was added to the Resuspension Solution.*

Add **40 ml of the Resuspension/RNase A Solution** to the bacterial pellet and completely resuspend by pipetting up and down or vortexing. Incomplete resuspension can result in poor recovery of plasmid DNA. Combine suspensions into a single 500 ml container if more than one centrifuge bottle was used for pelleting cells.

4. Lyse cells

Lyse the resuspended cells by adding **40 ml of Lysis Solution**. Immediately mix the contents by gently inverting 10–12 times. Let the mixture sit for 3–5 minutes until it becomes clear and viscous.

Do not shake or vortex. Harsh mixing will shear genomic DNA and may contaminate the final recovered plasmid DNA.

Do not allow lysis to proceed longer than 5 minutes. *Prolonged alkaline lysis may permanently denature the supercoiled plasmid DNA and may render it unsuitable for use in downstream applications.*

5. Neutralize lysate

Important Reminder: *Confirm that Neutralization Solution is chilled to 2–8 °C.*

Neutralize the lysed cells from Step 4 by adding **40 ml of chilled Neutralization Solution**. Gently invert the tube 10–12 times to ensure thorough mixing. A white aggregate (cell debris, proteins, lipids, SDS, and chromosomal DNA) will form.

Note: The use of Neutralization Solution that is not pre-chilled may result in increased endotoxin level in final plasmid preparation.

6. Prepare lysate for filtering

Add the contents of **one package of Lysate Clearing Agent** to the neutralized lysate from Step 5. Invert gently several times to ensure thorough mixing. **Incubate at room temperature for 5 minutes.**

Note: Omitting the 5 minutes incubation may result in increased level of endotoxin in final plasmid preparation.

7. Prepare filter unit

Attach the GenElute HP Megaprep Filter(s) to a sterile 250 ml collection bottle with a 45 mm neck, and attach the collection bottle to the vacuum source.

For convenience, this step may be performed before starting the procedure or at any point prior to this step.

8. Filter lysate

Mix lysate again gently, pour the lysate mixture into the top of the assembled filtration unit (Step 7), and immediately apply vacuum. After lysate passes through, turn off vacuum. Disassemble the filtration unit and discard the Megaprep Filter.

9. Add Binding Solution

Add **50 ml Binding Solution** to the cleared lysate in the collection bottle. Gently swirl the collection bottle to mix.

10. Bind plasmid DNA

Important Reminder: *Do not allow the Column to dry during this step.*

Transfer the mixture from Step 9 to the prepared Binding Column(s) in step 2 and apply vacuum until all liquid passes through.

It is acceptable to leave a residual amount of lysate/binding mixture on the column prior to continuing. Drying will significantly reduce the flow rate in step 11, and could result in a 2-3 fold increase in the amount of vacuum time required to complete step 11.

Note: If the Binding Column was not prepared as described in step 2, prepare it now and **wait 10 minutes** before loading the cleared lysate. Failure to wait 10 minutes may result in reduced yield. The cleared lysate may be stored at 2–8 ºC until the column is ready.

11. Apply Wash Solution 1

Add **40 ml of Wash Solution 1** to the column(s) and allow it to pass through.

12. Apply Wash Solution 2

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

Add **60 ml of Wash Solution 2** to the column and allow it to pass through.

13. Dry column

Important Reminder: *Make certain the vacuum level is equal to or greater than 500 mbar (refer to Appendix 2 for unit conversions).*

Following the wash steps, leave the vacuum on for **20 minutes** to dry the column. *If **more than one column** is on the vacuum manifold, dry the columns for **30 minutes**.*

It is important to completely dry the column to prevent ethanol contamination and allow efficient elution in the final preparation. Depending on the strength of the vacuum source, it may be necessary to increase the vacuum time.

14. Elute plasmid DNA

Attach the Binding Column to a red VacCap and connect to a Collection Tube and the vacuum source. Refer to the Elution Options table below to determine which elution volume is appropriate.

For maximum recovery of plasmid DNA: Add **20 ml of Endotoxin-Free Water** to the column and apply vacuum for **5 minutes**. Turn vacuum off and discard the Binding Column.

For maximum concentration of plasmid DNA: This elution protocol employs two separate volumes of Endotoxin-Free Water. First, add **5 ml of Endotoxin-Free Water** and apply vacuum for **2 minutes**. Then, add another **5 ml of Endotoxin-Free Water** and continue applying the vacuum for an additional **5 minutes**. Turn vacuum off and discard the Binding Column.

Elute Options

Volume Applied	Volume Recovered	Relative Yield	Relative Concentration
20 ml	14–16 ml	100%	100%
2 x 5 ml	4–6 ml	90%	250–300%

The plasmid DNA is present in the eluate and is ready for immediate use, concentration by precipitation, short-term storage at 2–8 °C or long-term storage at –20 °C.

DNA Concentration

Important Reminder: *Alcohol precipitation is only necessary if a more concentrated plasmid preparation is desired.*

Transfer the recovered eluate to an endotoxin-free (pyrogen-free) centrifuge bottle. Please note that the provided Collection Tubes should not be centrifuged above 5000 x *g*.

Add **0.1 volumes** of 3 M Sodium Acetate Buffer Solution, pH 5.2 and **0.7 volumes** of isopropanol to the recovered plasmid. Mix well by inversion and centrifuge at 12,000–15,000 x *g* for 30 minutes, or 5000 x *g* for 60 minutes at 4 °C. Remove the supernatant, being careful not to disturb the pellet. Rinse the DNA pellet with **5–10 ml** of 70% ethanol and centrifuge at 12,000-15,000 x *g* for 10 minutes, or 5000 x *g* for 15 minutes. 10 minutes. Carefully decant the supernatant. Air-dry the pellet until all residual ethanol has evaporated. Resuspend the DNA pellet in endotoxin-free water.

DNA Quantitation

Recovery and purity of the plasmid DNA may be determined by spectrophotometric analysis. The ratio of absorbance at $(A_{260}–A_{320})/(A_{280}–A_{320})$ should be 1.8–2.0. The A_{320} reading corrects for any background absorbance, including that caused by silica fines in the final product. These fines are common in silica-based systems and will have no effect on downstream applications. To remove silica fines, spin the eluate at 5000 x *g* for 10-15 minutes and recover the supernatant. The size and quality of the DNA may be determined by agarose gel electrophoresis or pulse field gel 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).

Troubleshooting Guide

Lysate clogs Lysate Filter(s) in Step 8	**Cause** — The bacterial culture was too dense. Cell mass value too high. **Solution** — Allow longer time to pass the lysate through completely. Do not grow cultures beyond recommended cell density. See Preparation Instructions, step 2, page 3.
Poor or no plasmid DNA recovery	**Cause** — Cells overgrown or undergrown. **Solution** — Confirm cell density by taking absorbance at 600 nm (A_{600}). A healthy culture generally reaches an A_{600} of 2-4 in LB media and an A_{600} 3-6 in TB media.
	Cause — Too many or few cells harvested. **Solution** — Confirm that an appropriate cell mass was used. See Preparation Instructions, step 2, page 3.
	Cause — Starting culture is too old. **Solution** — Streak a fresh plate from a freezer stock. Pick a single colony and prepare a new culture.
	Cause — The culture was not processed immediately. **Solution** — Fresh overnight cultures should be used for plasmid preparations. If cultures must be stored before use, pellet the cells, remove the culture medium, and store wet pellets at –70 °C.
	Cause — Plasmid replication is poor. **Solution** — Confirm that the cells were grown in the appropriate medium with a selective antibiotic under optimized conditions.
	Cause — Antibiotic activity is insufficient. **Solution** — Confirm that the appropriate amount of fresh antibiotic was present during growth of culture. Most antibiotics are light sensitive and degrade during long term storage at 2–8 °C.
	Cause — Wash Solution 2 is too concentrated. **Solution** — Confirm that Wash Solution 2 was diluted with the specified volume of ethanol. Keep the bottle tightly capped between uses to prevent evaporation.
	Cause — Alkaline lysis exceeded 5 minutes. **Solution** — Prolonged alkaline lysis may permanently denature plasmid DNA. Do not allow lysis to exceed 5 minutes.
	Cause — Precipitation of cell debris is incomplete. **Solution** — Thoroughly mix the lysate following the addition of the chilled Neutralization Solution.

Poor or no plasmid DNA recovery	**Cause** — Lysis is incomplete. **Solution** — Too many cells harvested. See Preparation Instructions, step 2, page 3. Lyse cells 3–5 minutes until the mixture becomes clear and viscous. See Step 4 in Procedure.
	Cause — Vacuum level is too low. **Solution** — Vacuum source should attain ≥500 mbar (refer to Appendix 2 for unit conversions).
Absorbance readings do not accurately reflect the quantity of plasmid	**Cause** — The plasmid DNA is contaminated with RNA; RNase A treatment is insufficient. **Solution** — Confirm that RNase A Solution was added to the Resuspension Solution prior to first use. Store the Resuspension/RNase A Solution at 2–8 °C.
	Cause — The 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 or after the lysis reaction.
A_{260}/A_{280} ratio is too high or low	**Cause** — The background reading is too high or low high due to silica fines. **Solution** — Subtract background at A_{320} as described under DNA Quantitation. To remove silica fines, spin the eluted sample at 5000 x *g* for 10 minutes and recover the supernatant.
	Cause — Wash Solution 2 is diluted with ethanol containing impurities. **Solution** — Check the absorbance of ethanol between 250 and 300 nm. Do not use ethanol with high absorbance. Traces of impurities may remain on the Binding Column after washing. Impurities may show up in the eluate and may contribute to the absorbance of the final product.
Additional band migrates behind supercoiled plasmid during electrophoresis	**Cause** — Some of the supercoiled plasmid DNA has become nicked. **Solution** — Plasmid DNA that has been nicked will run slower than supercoiled DNA during electrophoresis. A small amount of this species of DNA is common and is suitable for downstream applications.
Additional band migrates behind supercoiled plasmid during electrophoresis	**Cause** — Some of the supercoiled plasmid DNA has become permanently denatured. **Solution** — Do not allow the lysis reaction to proceed longer than 5 minutes. Permanently denatured plasmid DNA will migrate ahead of the supercoiled DNA and may not be suitable for downstream applications.

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 55–65 °C until dissolved. Cool to room temperature prior to use.
	Cause — The plasmid DNA is permanently denatured. **Solution** — Do not allow the lysis reaction to proceed longer than 5 minutes. Prolonged alkaline lysis may permanently denature plasmid DNA.
	Cause — DNA concentration is too low. **Solution** — Precipitate the DNA and resuspend in a desired volume as described under DNA Concentration.
	Cause — Ethanol is present in the final elution. **Solution** — Increase the drying time of the column following the second wash. Vacuum source should attain >500 mbar (refer to *Appendix 2* for unit conversions).
	Cause — High salt concentration in final elution. **Solution** — Confirm that Wash Solution 2 followed Wash Solution 1. Wash Solution 2 removes residual salt and other impurities from the column. Precipitate the plasmid DNA as described under DNA Concentration.

Related Products	Product Codes	Related Products	Product Codes
3-Way Stopcock	**Z28,647-8**	Precast Agarose Gels, 1.0%, 8 well	**P 5472**
T-Tubing Connectors	**Z17,848-9**	Gel Loading Solution	**G 2526**
Kimwipes® Disposable Wipers	**Z18,895-6**	DirectLoad™ Wide Range DNA Marker	**D 7058**
LB Broth, Sterile Liquid Media	**L 2542**	Ethidium bromide, aqueous, 10 mg/ml	**E 1510**
TB Broth, Sterile Liquid Media	**T 5574**	TAE Buffer (10X Concentrate)	**T 9650**
Water, Molecular Biology Reagent	**W 4502**	TBE Buffer (10X Concentrate)	**T 4415**
Endotoxin-Free Water	**W3500**	Escort II Transfection Reagent	**L 6037**
3M Sodium Acetate Buffer Solution, pH 5.2	**S 7899**	Escort V Kit-Enhanced	**E 1029**
Isopropanol	**I 9030, I 0398 or I 9516**		

Appendix 1: Centrifuge Speed Conversion Table

All centrifugation speeds are given in units of gravity (*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.

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

Centrifuge	Rotor	Type*	Radius (cm)	RPM at 3000 x *g*	RPM at 5000 x *g*
Beckman					
Allegra 6	GH-3.8	SB	20.4	3,631	4,688
Allegra 21(R)	S4180	SB	16.1	4,081	5,268
Allegra 64	F0485	FA	9.0	N/A**	N/A
	F0685	FA	9.7	N/A	N/A
TJ-25	TS-5.1-500	SB	19.0	3,756	4,849
	TA-10-250	FA	13.7	N/A	N/A
Rotors for older Beckman centrifuges	JA-10	FA	15.8	N/A	N/A
	JA-14	FA	13.7	N/A	N/A
	JA-20	FA	10.8	N/A	N/A
	JS-13	FA	14.0	N/A	N/A
IEC	215	SB	13.0	4,537	5,857
MP4(R)	224	SB	35.9	2,733	3,528
PR-7000M	966	SB	24.5	3,310	4,274
B22M	877	FA	12.6	N/A	N/A
Sorvall	HB-4	SB	14.7	4,277	5,522
	HB-6	SB	14.6	4,284	5,531
	HS-4	SB	17.2	3,948	5,097
	SH-80	SB	10.1	5,142	6,639
	GSA	FA	14.5	N/A	N/A
	SA-300	FA	9.7	N/A	N/A
	SA-600	FA	12.9	N/A	N/A
	SE-12	FA	9.3	N/A	N/A
	SL-50T	FA	10.7	N/A	N/A
	SS-34	FA	10.7	N/A	N/A

*SB = swinging bucket; FA = fixed angle
**N/A = not appropriate for application

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

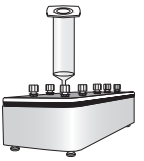
Appendix 2: Vacuum Pressure Conversion Table

All vacuum pressures are given in millibars (mbar). Please refer to Table 2 for information on converting millibars (mbar) to other pressure units.

Table 2. Conversion of millibars (mbar) to Other Pressure Units

Pressure Unit	500 mb equivalent
Inches of mercury (inch Hg)	14.8
Millimeters of mercury (mm Hg)	375
Pounds per square inch (psi)	7.25
Atmospheres (atm)	0.49
Kilopascals (kPa)	50
Torrs (Torr)	375

Experienced User Protocol



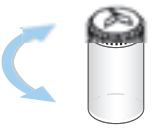
❒ **Preparation**:

- Add 1.25 ml RNase A to the Resuspension Solution
- Add 300 ml of 95–100% Ethanol to Wash Solution 2 (75 ml)
- Chill the Neutralization Solution to 2–8 °C

1 Harvest Bacteria

❒ Pellet **200–1000 ml** of an overnight culture at 5000 x *g*, 10 minutes. Discard supernatant.

2 Prepare Column



❒ Place Megaprep Binding Column(s) on vacuum manifold

❒ Add **40 ml of Column Preparation Solution** to Megaprep Binding Column(s). Apply vacuum until all liquid passes through column.

3 Resuspend & Lyse Bacteria

❒ Resuspend cells in **40 ml of Resuspension Solution**. Pipette up and down to mix.

❒ Add **40 ml of Lysis Solution** and gently invert 6–8 times to mix. Do not vortex. Allow to clear, 3–5 minutes.

4 Prepare Cleared Lysate

❒ Add **40 ml of chilled Neutralization Solution** to the lysed cells and gently invert 6–8 times to mix.

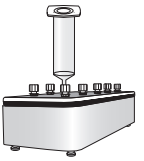
❒ Attach the Lysate Filter to a 45 mm neck bottle (≥250 ml).

❒ **Empty entire bag of Lysate Clearing Agent** into lysate and gently invert 6–8 times to mix. **Incubate 5 Minutes at RT**.

❒ Add lysate to assembled Lysate Filter and apply the vacuum to clear the lysate of cell debris.

❒ Add **50 ml of Binding Solution** to the bottle containing the cleared lysate and gently swirl to mix.

5 Bind Plasmid DNA to Column



❒ Add cleared lysate to prepared Megaprep Binding Column, apply vacuum, and allow all lysate to pass through.

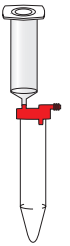
6 Wash to Remove Contaminants

❒ Add **40 ml of Wash Solution 1** to the Binding Column(s) and allow it to pass through.

❒ Add **60 ml of Wash Solution 2** to the Binding Column and allow it to pass through.

❒ Leave the vacuum on for **20–30 minutes to dry** the Binding Column(s).

7 Elute Purified Plasmid DNA



❒ Attach a VacCap to a 50-ml collection tube, provided.

❒ Attach the Binding Column(s) to the VacCap.

❒ **For Maximum Yield of Plasmid DNA**: Add **20 ml of Endotoxin-Free Water** to Binding Column(s) and apply vacuum for 5 minutes.

❒ **For Maximum Concentration of Plasmid DNA**: Add **5 ml of Endotoxin-Free Water**. Apply vacuum for 2 minutes. Add another **5 ml of Endotoxin-Free Water** and continue vacuuming for 5 minutes.

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