User Guide

Catalog Nos.
NA0400
NA0400S
NA0410

GenElute™ HP Endotoxin-Free Plasmid Maxiprep Kit

sigma.com

Ordering Information

Cat. No.	Product Description	Pkg Size
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

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
NA0500	GenElute HP Plasmid Megaprep Kit	5 preps
NA0600	GenElute HP Endotoxin-Free Plasmid Megaprep Kit	5 preps
NA0800	enElute HP Select Plasmid Gigaprep Kit	5 preps

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GenElute™ HP Endotoxin-Free Plasmid Maxiprep Kit

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Product Description

Endotoxins are a common contaminant in plasmid preparations that can reduce transfection efficiencies in endotoxin-sensitive eukaryotic cell lines. The GenElute™ HP Endotoxin-Free Plasmid Maxiprep Kit offers a simple and rapid method for isolating endotoxin-free plasmid DNA from recombinant *E. coli* cultures. The kit uses a vacuum format with a filter column for the rapid clearing of the bacterial lysate and a silica column for capturing plasmid DNA. Up to 1.2 mg of plasmid DNA with < 0.1 Endotoxin Units/μg can be isolated from an overnight culture grown in Luria Broth (LB) medium in about 40 minutes. Note that the plasmid yield and endotoxin levels will vary depending on the strain, the plasmid, and the culture medium used.

An overnight recombinant *E. coli* culture is harvested by centrifugation and subjected to a modified alkaline-SDS lysis. The lysate is clarified 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 on silica, while endotoxins are prevented from adsorbing to the membrane. Two wash steps remove contaminants. 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 transfection, transformation, restriction digestion, ligation, sequencing and PCR.

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

Reagents Provided	Catalog No.	NA0400S 4 Preps	NA0400 10 Preps	NA0410 25 Preps
Column Preparation Solution	**C2112**	60 ml	225 ml	2 x 225 ml
RNase A Solution	**R6148**	1.5 ml	1.5 ml	2.5 ml
Resuspension Solution	**R1149**	60 ml	150 ml	375 ml
Lysis Solution	**L1912**	60 ml	150 ml	375 ml
Neutralization Solution	**N7411**	60 ml	150 ml	375 ml
Binding Solution	**B9560***	45 ml	115 ml	280 ml
Wash Solution 1	**W0263**	60 ml	150 ml	375 ml
Wash Solution 2	**W4639**	12 ml	30 ml	75 ml
Endotoxin-Free Water	**2107**	50 ml	50 ml	100 ml
GenElute HP Endotoxin-Free Maxiprep Filter	**H3538**	4	10	25
VacCap	**R4778**	5	10	25
GenElute HP Maxiprep Binding Column	**G4917**	4	10	25
Collection Tubes — 50 ml	**C4353**	8	20	50

*** 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.**

Equipment and Reagents Required But Not Provided

- Centrifuge capable of 5000 x *g*
- Centrifuge with a swinging bucket rotor capable of 3000 x *g*
- Vacuum source capable of ≥500 mbar. See Appendix 2 for unit conversions.
- Vacuum manifold, Catalog No. **VM20**
- Ethanol (95–100%), Catalog Nos. **E7148**, **E7023**, or **459836**

Precautions and Disclaimer

The GenElute HP Endotoxin-Free Plasmid Maxiprep Kit is for research use only and is 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 to 5 ml LB 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 volume of LB medium and incubate at 37 °C for 12 to 16 hours while shaking at 250–300 rpm.

A healthy culture grown in LB will generally reach an absorbance at 600 nm of between 2 and 4. Cultures grown in rich media such as TB are not recommended for use with this kit.

2. Choosing the correct culture volume

Use of **150 ml** of culture 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 and may cause a very fine flocculent after neutralization that could cause clogging during filtration. 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.

2. Choosing the correct culture volume (cont'd)

For best results, we recommend using a volume of culture based on cell mass. A total cell mass of 200–600 is recommended but **a cell mass of 450 is typically optimal**. The optimal volume of culture to use can be determined by measuring the absorbance of the overnight culture at 600 nm (A_{600}) and using the formula below:

$$\text{Volume}_{\text{optimal}} = \frac{450}{A_{600}}$$

3. Mix reagents thoroughly

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

4. Prepare Resuspension Solution/RNase A

Spin the tube of RNase A Solution (**R6148**) briefly to collect the solution in the bottom of the tube. Add the amount of RNase A Solution listed in the table below to the bottle of Resuspension Solution (**R1149**). Store at 2–8 °C.

Kit Size	Volume of RNase A Solution to Add
4 preps	300 µl
10 preps	750 µl
25 preps	1.9 ml

5. Prepare Wash Solution 2

Add the amount of 95–100% ethanol listed in the table below 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 the ethanol.

Kit Size	Volume of Ethanol to Add
4 preps	48 ml
10 preps	120 ml
25 preps	300 ml

6. Chill Neutralization Solution

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

Convenient stopping points

Procedure

All steps are carried out at room temperature. When using a vacuum, make certain the vacuum level is equal to or greater than 500 mbar (refer to Appendix 2 for unit conversions).

Step 1:

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

Step 2 and step 8:

Do not prepare Binding Column in Step 2. Instead perform Steps 1 and 3-8. Now cleared lysate containing Binding Solution can be stored overnight at 2-8 ºC 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,** then load the cleared lysate containing Binding Solution to the column and follow the procedure to finish the DNA preparation.

1. Harvest cells

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

Pellet **150 ml of an overnight culture** by centrifugation at **5000 x *g* for 10 minutes** and discard the supernatant.

2. Prepare Binding Column

Place a GenElute HP Maxiprep Binding Column onto the Sigma VM20 vacuum manifold. Add **12 ml of Column Preparation Solution** to the Binding Column, apply the vacuum and allow it to pass through. Then turn vacuum off until step 9.

3. Resuspend cells

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

Add **12 ml of 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.

4. Lyse cells

Lyse the resuspended cells by adding **12 ml of Lysis Solution**. Immediately mix the contents by gently inverting 6 to 8 times. Let the mixture sit for 3 to 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. Prepare lysate filter

Place a VacCap firmly onto a Collection Tube. Attach a GenElute HP Endotoxin-Free Maxiprep Filter to the VacCap. Place this assembly into an appropriate holder.

6. Neutralize lysate

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

Neutralize the lysed cells from Step 3 by adding **12 ml of chilled Neutralization Solution** to the mixture and gently invert 6 to 8 times. Immediately pour the lysate into the filter assembly and **incubate for 5 minutes**. A white aggregate (cell debris, proteins, lipids, SDS, and chromosomal DNA) will form. *Do not add the Binding Solution until the lysate has been filtered in Step 7.*

7. Filter lysate

Important Reminder: *Do not remove the filter until the vacuum pressure is relieved.*

Filter lysate by attaching vacuum source to VacCap and applying vacuum. Allow lysate to filter through for at least 1 minute. Once the lysate has passed through, turn off vacuum and detach the vacuum source from the VacCap.

Some lysate may remain trapped in the white flocculent material. It is not necessary to recover this solution completely.

8. Add Binding Solution

Add **9 ml of Binding Solution** to the filtered lysate and gently invert 6–8 times to mix.

9. Bind Plasmid DNA

Important Tip: *Do not allow the lysate to pass below the surface of the binding material until all the lysate has been added to the column.*

Transfer the mixture from Step 8 to the prepared Binding Column with the vacuum on. Allow all the lysate to pass through.

The Binding Column will not accommodate the entire volume of lysate, so be careful not to overfill the column. If the column does empty before all the lysate is loaded, the remaining lysate will pass through the column very slowly. This may take several minutes, but will not have an effect on plasmid recovery or endotoxin levels.

VM20 Vacuum Manifold

10. Apply Wash Solution 1

Add **12 ml of Wash Solution 1** to the column and allow it to pass through.

11. Apply Wash Solution 2

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

Add **12 ml of Wash Solution 2** to the column and allow it to pass through.

12. Dry column

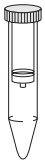
Important Reminder: *Make certain the vacuum level is greater than or equal to 500 mbar (refer to Appendix 2 for unit conversions)*

Following the wash steps, leave the vacuum on for at least **10 minutes** to dry the column. *If **more than 6 columns** are on the vacuum manifold, dry for at least **20 minutes**.*

It is important to completely dry the column to prevent the 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.

Remove any Wash Solution remaining on the inside of the column with a Kimpwipes®.

13. Elute plasmid DNA



Transfer the Binding Column to a clean 50 ml Collection Tube. Add **3 ml of Endotoxin-Free Water** to the column. Refer to Elution Options table below to determine which centrifugation speed is appropriate.

For maximum recovery of plasmid: Centrifuge the column/collection tube unit in a swinging bucket rotor at **3000 x *g* for 5 minutes**.

For maximum concentration of plasmid: Centrifuge the column/collection tube unit in a swinging bucket rotor at **1000 x *g* for 5 minutes**.

Centrifugation Speed	Typical Volume Recovered	Relative Yield	Relative Concentration
3000 x *g*	2.5 ml	100%	100%
1000 x *g*	1.2 ml	80%	175%

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

Add **0.1 volumes** of 3.0 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 ≥15,000 x *g* at 4 °C for 30 minutes. Decant the supernatant, being careful not to disturb the pellet. Rinse the DNA pellet with **1.5 ml** of 70% ethanol and centrifuge as before for 10 minutes. Carefully decant the supernatant and air-dry the pellet until the residual ethanol has evaporated. Resuspend the DNA pellet in the desired volume of 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 to 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 should not effect most downstream applications. To remove silica fines, spin the elute at 5000 x g (italic 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.; Doly, J. A rapid alkaline extraction procedure for screening recombinant plasmid DNA. *Nucleic Acids Res.*, **1979**, *7*, 1513–1522.
2. Vogelstein, B.; Gillespie, D. Preparative and analytical purification of DNA from agarose. *Proc. Natl. Acad. Sci. USA*, **1979**, *76*, 615–619.

Troubleshooting Guide

Lysate is not clear after filtration; binding column becomes clogged	**Cause** — Cells have been stored improperly before use. **Solution** — If the culture cannot be processed immediately, pellet the cells and store at –70 °C.
Binding Column appears clogged following the addition of the lysate	**Cause** — The Binding Column was allowed to empty before all the lysate was loaded onto the column. **Solution** — Do not allow the lysate to pass below the surface of the binding material until all the lysate has been added to the column. If the column does empty before all the lysate is loaded, the remaining lysate will pass through the column very slowly. This may take several minutes but should not have an effect on plasmid recovery or endotoxin levels.
Elution volume recovery is greater than 3 ml	**Cause** — Binding Column was not dried sufficiently after the second wash step. **Solution** — Increase the drying time of the column to 20 minutes following the second wash. Vacuum source should attain ≥500 mbar (refer to Appendix 2 for unit conversions).
Poor or no plasmid DNA recovery	**Cause** — Cells overgrown or undergrown. **Solution** — Confirm cell density by taking absorbance at 600 nm. See Preparation Instructions 2.
	Cause — Too many or few cells harvested. **Solution** — Confirm that an appropriate cell mass was used. See Preparation Instructions 2.
	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 — 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.

Poor or no plasmid DNA recovery	**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.
	Cause — Lysis is incomplete. **Solution** — Too many cells harvested. See Preparation Instructions 2. Lyse cells 3 to 5 minutes until the mixture becomes clear and viscous.
	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 high. **Solution** — Subtract background at A_{320} as described under DNA Quantitation.
	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 (covalently opened) 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 ahead of 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 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 to 20 minutes 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	Catalog No.	Related Products	Catalog No.
Kimwipes® Disposable Wipers	**Z188956**	Gel Loading Solution	**G 2526**
LB Broth, Sterile Liquid Media	**L 2542**	DirectLoad™ Wide Range DNA Marker	**D 7058**
Water, Molecular Biology Reagent	**W 4502**	Ethidium bromide, aqueous, 10 mg/ml	**E 1510**
Endotoxin-Free Water	**W3500**	TAE Buffer (10X Concentrate)	**T 9650**
3M Sodium Acetate Buffer Solution, pH 5.2	**S 7899**	TBE Buffer (10X Concentrate)	**T 4415**
Isopropanol	**I 9030, I 0398 or I 9516**	Escort II Transfection Reagent	**L 6037**
Precast Agarose Gels, 1.0%, 8 well	**P 5472**	Escort V Kit-Enhanced	**E 1029**

Appendix 1: Centrifuge Speed Conversion Table

All centrifugation speeds are given in units of gravity (*g*). Please refer to Table 1 or 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

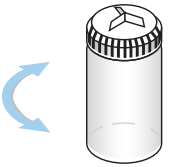
Pressure Unit	500 mb equivalent
Inches of mercury (inch Hg)	14.8
Millimeters of mercury (mm Hg)	375
Pounds per square inch (psi)	7.25
Atmospheres (atm)	0.49
Kilopascals (kPa)	50
Torrs (Torr)	375

Experienced User Protocol

- **Preparation**: See Technical Bulletin for Details
  - Add RNase A to Resuspension Solution
  - Add Ethanol to Wash Solution 2
  - Chill Neutralization Solution

1 Harvest Bacteria

- Pellet **150 ml** of an overnight culture at 5000 x *g*, 10 minutes. Discard supernatant.

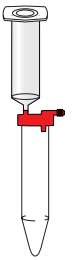


2 Prepare Column

- Place a Maxiprep Binding Column onto the Sigma VM20 vacuum manifold. Add **12 ml** of Column Preparation Solution to the binding column and vacuum through.

3 Resuspend & Lyse Bacteria

- Resuspend cells in **12 ml** of Resuspension/RNase Solution. Pipet up and down, or vortex to mix.
- Add **12 ml** of Lysis Solution and gently invert 6–8 times to mix. Do not shake or vortex. Allow to clear, 3–5 minutes.



4 Prepare Cleared Lysate

- Neutralize lysate by adding **12 ml** of chilled Neutralization Solution and gently invert 6–8 times to mix.
- Prepare lysate filter by placing a VacCap onto a collection tube and attaching an Endotoxin-Free Maxiprep Filter to the VacCap.
- Immediately pour the lysate into the filter assembly and **incubate for 5 minutes**.
- Filter lysate by attaching vacuum source to the VacCap and applying vacuum.
- Add **9 ml** of Binding Solution to the filtered lysate and gently invert 6–8 times.

5 Bind Plasmid DNA to Column

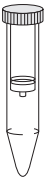
- Transfer lysate to the prepared column with the vacuum on. **Do not allow the lysate to pass below the surface of the binding material until all the lysate has been added to the column**.

6 Wash to Remove Contaminants

- Add **12 ml** of Wash Solution 1 and vacuum through.
- Add **12 ml** of Wash Solution 2 and vacuum through.
- Leave the vacuum on for at least **10 minutes** to dry the column. If more than six columns are on the manifold, dry for at least 20 minutes.

7 Elute Purified Plasmid DNA

- Transfer the Binding Column to a collection tube.
- Add **3 ml** of Endotoxin-Free Water to the Binding Column.
- **For maximum yield of Plasmid DNA**: centrifuge the column/collection tube unit in a swinging bucket rotor at 3000 x *g* for 5 minutes.
- **For maximum concentration of Plasmid DNA**: centrifuge the column/collection tube unit in a swinging bucket rotor at 1000 x *g* for 5 minutes.



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