User Guide

Catalog Nos.
NA0800

GenElute™ HP Select Plasmid Gigaprep Kit

sigma.com

Ordering Information

Catalog Number	Product Description	Pkg Size
NA0800	GenElute HP Select Plasmid Gigaprep Kit	5 preps

Related Products

Catalog Number	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
NA0600	GenElute HP Endotoxin-Free Plasmid Megaprep Kit	5 preps

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GenElute HP Select Plasmid Gigaprep Kit

Table of Contents

Product Description 2
Precautions and Disclaimer 3
Storage and Stability 3
Preparation Instructions 3
Procedure 4
DNA Concentration 7
DNA Quantitation 7
References 7
Troubleshooting Guide 8
Appendix 1: Centrifuge Speed Conversion Table 11
Appendix 2: Vacuum Pressure Conversion Table 12
Experienced User Protocol 13

Product Description

The GenElute HP Select Plasmid Gigaprep Kit offers a simple, rapid, and cost-effective method for isolating endotoxin-free plasmid DNA from recombinant *E. coli* cultures. Up to 14 mg of high copy plasmid DNA with <0.1 endotoxin unit/µg DNA can be recovered from *E. coli* cultures in 1.2–2.5 liters Luria Broth (LB) medium or 600 mL–1.2 liters of Terrific Broth (TB) medium in less than 2.0 hours after harvesting the cells. Note that the actual yield depends 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 cleared by filtration followed by the addition of a binding solution 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 with three wash steps Finally, the bound DNA is eluted in endotoxin-free water.

The recovered plasmid DNA is predominantly in its supercoiled form. Genomic DNA and RNA are below detectable levels as determined 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.

Reagents Provided	Cat. No.	Quantity
Resuspension Solution	**R1149**	600 mL
RNase A Solution	**R6148**	4 mL
Lysis Solution	**L1912**	600 mL
Lysate Clearing Agent	**L4667**	5 pks
Neutralization Solution	**N7411**	600 mL
Binding Solution	**B9560**	525 mL
Column Preparation Solution	**C2112**	3 × 225 mL
EndoCleaning Solution	**E1656**	250 mL
Wash Solution 1	**W0263**	900 mL
Wash Solution 2	**W4639**	1 × 200 mL 1 × 75 mL
Endotoxin-free water	**W3391**	500 mL

Equipment Provided	Cat. No.	Quantity
GenElute HP Gigaprep Filters	**G6044**	5 each
GenElute HP Pro Gigaprep Binding Columns	**G3671***	5 each

*Polypropylene column housing is now incorporated into the GenElute HP Gigaprep Binding Columns (Product code G6419). This improved product, GenElute HP Pro Gigaprep Binding Columns (Product code G3671), will replace G6419. The performance and functionality of G3671 and G6419 are equivalent.

Equipment and Reagents Required But Not Provided

- Centrifuge capable of 5000 × *g*
- Centrifuge bottle with ≥500 mL capacity
- Vacuum source capable of ≥500 mbar (refer to Appendix 2 for unit conversions)
- Ethanol (95–100%), Catalog Nos. **E7148**, **E7023**, or **459836**
- Clean 500 and 1000 mL bottles with 45 mm necks, Catalog Nos. **CLS1395500** (500 mL); **CLS13951L** (1000 mL), 1 of each size per preparation
- 100 or 150 ml 45 mm neck Endotoxin-free receiver bottle, such as Corning (CLS8388; CLS431175), Nalgene Nunc (Z358231) and Millipore 250 mL Stericup™ receiver flask (Z717541), 1 per preparation

Storage and Stability

Store the kit at room temperature. The optimal temperature for long-term storage of the kit is 18–25 °C. After the RNase A Solution is added to the Resuspension Solution, store at 2–8 °C. The Neutralization Solution may 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 A_{600} of 2–4 in LB media or an A_{600} 3–6 in TB medium.

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 1.2 - 2.5 liter of LB medium or 600 - 1.2 liters of 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 3500-6000 for LB culture or 4500 for TB culture is recommended, but a **cell mass of 4500 in LB is typically optimal**. The optimal volume of culture to use can be calculated using the formula below:

$$\text{Volume}_{\text{optimal}} = \frac{4500}{A_{600}}$$

Pellet wet weight is another good factor to determine the amount of culture to use. A wet pellet weight of 12–18 g per preparation is recommended.

3. Mix Reagents Thoroughly

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

4. Prepare Resuspension/ RNase A Solution

Add 3.0 mL of RNase A Solution (**R6148**) to the bottle of Resuspension Solution (**R1149**) prior to initial use. Store at 2–8 °C.

5. Prepare Wash Solution 2

Add 800 mL of 95–100% ethanol to the larger bottle (200 mL size) of Wash Solution 2 (**W4639**) prior to initial use. Should additional quantities be needed, 300 mL of 95–100% ethanol can be added to the smaller bottle (75 mL size) of Wash Solution 2. After each use of Wash Solution 2, tightly cap the diluted wash solution to prevent ethanol evaporation.

6. Chill Neutralization Solution to 2–8 °C

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 ≥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. The vacuum may be turned on/off or remain on the entire time during washing steps based on researcher preference.

Convenient stopping points

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 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 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 continue to step 10 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 **1.2-2.5 liter of LB medium** or **600 mL to 1.2 liters of TB medium** of an overnight culture by centrifugation at 5000 × *g* for 10–15 minutes. Discard the supernatant.

2. Prepare Binding Column

Attach the GenElute HP Gigaprep Binding Column to a clean 45 mm neck 1000 mL collection bottle (user provided) and connect the unit to the vacuum source.

Prepare the Binding Column by adding **100 mL** of **Column Preparation Solution** to the Binding Column. Apply vacuum until all liquid passes through. Turn off the vacuum and set the unit aside until step 10.

Note: Do not use a clamp to hold the Binding Column. Clamp tightening can break the vacuum seal. If the column-collection bottle assembly is unsteady, clamp the collection bottle.

3. Resuspend Cells

Important Reminder: *Verify that 3.0 mL RNase A Solution was added to the Resuspension Solution.*

Add **100 mL** of the **Resuspension/RNase A Solution** to the bacterial pellet and completely resuspend by pipetting up and down, vortexing or shaking on a shaker at 200 rpm for 5–10 minutes.

Incomplete resuspension can result in poor recovery of plasmid DNA. Combine suspended bacteria into a single centrifuge bottle if more than one centrifuge bottle was used for pelleting cells.

4. Lyse Cells

Lyse the resuspended cells by adding **100 mL** of **Lysis Solution**. Immediately mix the contents by gently inverting 10 to 15 times.

Do not shake or vortex. Harsh mixing will shear genomic DNA and may contaminate the final recovered plasmid DNA. Let the mixture sit for 3–5 minutes until it becomes clear and viscous.

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 ℃.*

Neutralize the lysed cells by adding **100 mL** of **chilled Neutralization Solution**. Gently shake the bottle 10 to 15 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. Gently shake 6–10 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 Gigaprep Lysate filter to a clean 45 mm neck 500 mL collection bottle (user provided) and connect the unit to the vacuum source.

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

Note: Do not use a clamp to hold the Filtration Column. Clamp tightening can break the vacuum seal. If the column-collection bottle assembly is unsteady, clamp the collection bottle.

8. Filter Lysate

Mix lysate again gently. Pour the lysate mixture into the top of the filtration unit assembled in step 7 and immediately apply vacuum. After the lysate passes through, turn off the vacuum. Disassemble the lysate filtration unit and discard the Gigaprep Lysate Filter.

9. Add Binding Solution

Add **100 mL** of **Binding Solution** to the filtered lysate in the collection bottle. Gently swirl the collection bottle to mix.

10. Bind Plasmid DNA

Important Tip: *Turn off the vacuum as soon as the cleared lysate passing through the column slows from a steady flow to a drip.*

Reconnect the prepared Binding Column from step 2 to the vacuum source. Transfer the lysate/binding solution mixture from step 9 to the prepared Binding Column and apply vacuum until all liquid passes through.

Caution: Do not allow the column to dry during this step. 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 °C until the column is ready.

11. Apply EndoCleaning Solution

Add **40 mL** of **EndoCleaning Solution** to the column and allow it to pass through.

This step takes about 3–4 minutes.

12. Apply Wash Solution 1

Add **150 mL** of **Wash Solution 1** to the column and allow it to pass through.

This step takes about 2–3 minutes.

13. Apply Wash Solution 2

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

Add **200 mL** of **Wash Solution 2** to the column and allow it to pass through.

14. Dry Column

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

Following the wash steps, leave the vacuum on for **25–35 minutes** to dry the column.

Disassemble the Binding Column/bottle unit and discard the eluate properly. Check for and blot off residual liquid under the Binding Column.

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.

15. Elute Plasmid DNA

Attach the Binding Column to a 100 or 150 ml 45 mm neck Endotoxin-free receiver bottle (user provided) and connect the unit to the vacuum source. Refer to Table 1 to determine which elution volume is appropriate.

For maximum recovery of plasmid DNA:
Add **50 mL** of **Endotoxin-free water** to the column, **wait 1-2 minutes** and apply vacuum for **1–2 minutes**. Turn vacuum off and discard the Binding Column.

For maximum concentration of plasmid DNA:
Add **30 mL** of **Endotoxin-free water** to the column, **wait 1-2 minutes** and apply vacuum for **1–2 minutes**. Turn vacuum off and discard the Binding Column

Note: Do not use a clamp to hold the Binding Column. Clamp tightening can break the vacuum seal. If the column-collection bottle assembly is unsteady, clamp the collection bottle.

Table 1. Elution Options

Volume Applied	Volume Recovered	Relative Yield	Relative Concentration
50 mL	35 mL	100%	100%
30 mL	15 mL	70%	150–200%

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

DNA Concentration

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

Transfer the recovered eluate to an endotoxin-free (pyrogen-free) centrifuge bottle (user-provided).

Add **0.1 volume** of 3 M Sodium Acetate Buffer Solution, pH 5.2, and **0.7 volume** of isopropanol to the recovered plasmid. Mix well by inversion and centrifuge at 12,000–15,000 × *g* for 30 minutes, or 5000 × *g* for 60 minutes at 2–8 °C. Carefully remove the supernatant without disturbing the pellet. Rinse the DNA pellet with **15–20 mL** of 70% ethanol and centrifuge at 12,000–15,000 × *g* for 10 minutes, or 5000 × *g* for 15 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 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 eluate at 5000 × *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).
3. Ausubel, F. M., Brent, R., Kingston, R. E., Moore, D.D., Seidman, J.G., Smith, J.A. and Struhl, K. (eds). *Short protocols in molecular biology (3rd ed)*. John Wiley & Sons, New York, p. 1-3 (1997).

Troubleshooting Guide

Lysate clogs Lysate Filter(s) in Step 8	**Cause** — The bacterial culture was too dense or cell mass was 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** — Measure 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 was too old. **Solution** — Streak a fresh plate from a freezer stock. Pick a single colony and prepare a new culture.
	Cause — Plasmid replication was poor. **Solution** — Confirm that the cells were grown in the appropriate medium with a selective antibiotic under optimized conditions.
	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 — Antibiotic activity was insufficient. **Solution** — Confirm that the appropriate amount of fresh antibiotic was present during growth of the culture. Most antibiotics are light sensitive and degrade during long-term storage at 2–8 °C.
	Cause — Wash Solution 2 was 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.

Poor or no plasmid DNA recovery	**Cause** — Precipitation of cell debris is incomplete. **Solution** — Thoroughly mix the lysate following the addition of the chilled Neutralization Solution.
	Cause — Lysis was incomplete. **Solution** — Too many cells harvested. See "Preparation Instructions 2." Lyse cells 3–5 minutes until the mixture becomes clear and viscous.
	Cause — Vacuum level was too low. **Solution** — Vacuum source should attain ≥500 mbar (refer to Appendix 2 for unit conversions).
	Cause — Vacuum tubing collapsed. **Solution** — Change to appropriate vacuum tubing.
Absorbance readings do not accurately reflect the quantity of plasmid	**Cause** — The plasmid DNA is contaminated with RNA; RNase A treatment was 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 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 × *g* for 10–15 minutes and recover the supernatant.
	Cause — Wash Solution 2 was diluted with ethanol containing impurities. **Solution** — Check the absorbance of ethanol between 250 and 300 nm. Do not use ethanol with high absorbance readings. 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 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 to 50–60 minutes following the final 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."
Endotoxin level is high in plasmid DNA eluate	**Cause** — Construct grown in TB medium (especially low copy constructs). **Solution** — Grow the construct in LB media.
	Cause — Neutralization Solution not chilled. **Solution** — Chill or store the Neutralization Solution at 2–8 °C prior to use.
	Cause — Colony was passed from plate to plate many times. **Solution** — Start culture from frozen stock or retransform the plasmid DNA into *E. coli*, grow it in LB medium with appropriate amount of fresh antibiotic and save frozen stock immediately.
	Cause — Omitted the 5-minute incubation after adding Lysate Clearing Agent. **Solution** — After adding and mixing the Lysate Clearing Agent with the lysate, be sure to incubate the mix at room temperature for 5 minutes before filtering.

Related Products	Catalog No.	Related Products	Catalog No.
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**
KimwipesÒ Disposable Wipers	**Z18,895-6**	DirectLoadä 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 2 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 2. 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	3631	4688
Allegra 21(R)	S4180	SB	16.1	4081	5268
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	3756	4849
	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	4537	5857
MP4(R)	224	SB	35.9	2733	3528
PR-7000M	966	SB	24.5	3310	4274
B22M	877	FA	12.6	N/A	N/A

Centrifuge	Rotor	Type*	Radius (cm)	RPM at 3000 x *g*	RPM at 5000 x *g*
Sorvall	HB-4	SB	14.7	4277	5522
	HB-6	SB	14.6	4284	5531
	HS-4	SB	17.2	3948	5097
	SH-80	SB	10.1	5142	6639
	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 3 for information on converting millibars (mbar) to other pressure units.

Table 3. 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 3.0 mL RNase A to the Resuspension Solution (600 mL)
- Add 800 mL of 95–100% Ethanol to Wash Solution 2 (200 mL); should additional quantities be needed, add 300 mL of 95–100% Ethanol to Wash Solution 2 (75 mL)
- Chill the Neutralization Solution.

1 Harvest Bacteria

❒ Pellet **2000 mL** of an overnight culture at 5000 × *g*, 10 minutes. Discard supernatant.

2 Prepare Column

❒ Place a Gigaprep Binding Column onto a 45 mm neck bottle (1000 mL).

❒ Add **100 mL** of Column Preparation Solution to Gigaprep Binding Column, apply vacuum and allow it to pass through.

3 Resuspend & Lyse Bacteria

❒ Resuspend cells in **100 mL** of Resuspension RNase Solution. Pipette up and down or vortex to mix.

❒ Add **100 mL** of Lysis Solution and gently invert 10–12 times to mix. Do not vortex. Allow to clear, 3–5 minutes.

4 Prepare Cleared Lysate

❒ Add **100 mL** of Neutralization Solution to the lysed cells and gently shake 10–12 times to mix.

❒ **Empty** entire bag of Lysate Clearing Agent into lysate and gently shake several times to mix. **Incubate 5 minutes at RT.**

❒ Attach the Lysate Filter to a 45 mm neck bottle (≥500 mL).

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

❒ Add **100 mL** of Binding Solution to cleared lysate and swirl to mix.

5 Bind Plasmid DNA to Column

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

6 Wash to Remove Contaminants

❒ Add **40 mL** of EndoCleaning Solution to the Gigaprep Binding Column and allow it to pass through.

❒ Add **150 mL** of Wash Solution 1 to the Gigaprep Binding Column and allow it to pass through.

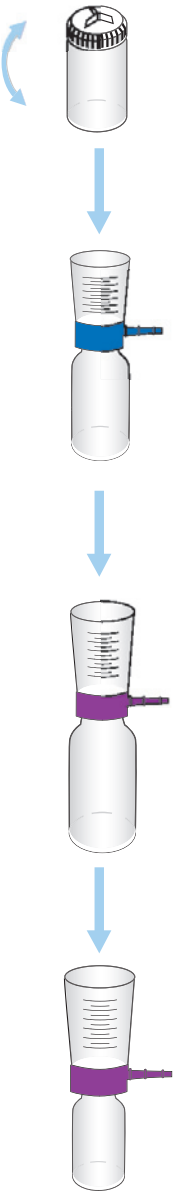
❒ Add **200 mL** of Wash Solution 2 to the Gigaprep Binding Column and allow it to pass through.

❒ Leave the vacuum on for 25–35 minutes to dry the Gigaprep Binding Column.

7 Elute Purified Plasmid DNA

❒ Attach the Gigaprep Binding Column to another sterile Endo-Free 45 mm neck bottle (150–250 mL).

❒ Add **30 or 50 mL** of Endotoxin-Free Water to Gigaprep Binding Column, **wait 1-2 minutes** and apply vacuum for 2–3 minutes.



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