Spin Format Protocol Modification

GenElute™ High Performance (HP) Endotoxin-Free Plasmid Maxiprep Kit

Product Codes **NA0400S, NA0400, NA0410**

TECHNICAL BULLETIN

Product Description
Endotoxins are a common contaminant in plasmid preparations that can reduce transfection efficiencies in 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 centrifuge 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 $\leq$ 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.

Reagents Provided	Product Code	NA0400S 4 Prep	NA0400 10 Prep	NA0410 25 Prep
Column Preparation Solution	C 2112	60 ml	225 ml	2 x 225 ml
RNase A Solution	R 6148	1.5 ml	1.5 ml	2.5 ml
Resuspension Solution	R 1149	60 ml	150 ml	375 ml
Lysis Buffer	L 1912	60 ml	150 ml	375 ml
Neutralization Solution	N 7411	60 ml	150 ml	375 ml
Binding Solution	B 1810	45 ml	115 ml	280 ml
Wash Solution 1	W 0263	60 ml	150 ml	375 ml
Wash Solution 2	W 4639	12 ml	30 ml	75 ml
Endotoxin-Free Water	210-7	50 ml	50 ml	100 ml
GenElute HP Endotoxin-Free Maxiprep Filter	H 3538	4	10	25
VacCap	R 4778	5	10	25
GenElute HP Maxiprep Binding Column	G 4917	4	10	25
Collection Tubes – 50 ml	C 4353	8	20	50

Equipment and Reagents Required But Not Provided
- Centrifuge capable of 5,000 x *g*
- Centrifuge with a swinging bucket rotor capable of 3,000 x *g*
- Ethanol (95-100%), Product Codes E 7148, E 7023, or 45,983-6
- 3 M Sodium Acetate Buffer Solution, pH 5.2 (optional), Product Code S 7899
- Isopropanol (optional), Product Codes I 9030, I 0398, or I 9516
- Additional 50 ml conical collection tubes

Spin Format Protocol Modification

Precautions and Disclaimer
The GenElute HP Endotoxin-Free Plasmid Maxiprep 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/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. Grow Bacterial Cultures
   For maximum plasmid recovery, begin with a single colony from a freshly streaked plate. Grow in LB medium containing the appropriate selective antibiotic at 37 °C. Shake vigorously at 250-300 RPM for overnight. 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. 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.

3. Prepare Resuspension Solution/RNase A
   Spin the tube of RNase A Solution (R 6148) briefly to collect the solution in the bottom of the tube. According to the table below, add the amount of RNase A Solution listed in the Table below to the bottle of Resuspension Solution (R 1149). Store at 2-8 °C.

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

4. Prepare Wash Solution 2
   According to the table below, add the appropriate amount of 95-100% ethanol to the bottle of Wash Solution 2 (W 4639) 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 prep	48 ml
10 prep	120 ml
25 prep	300 ml

5. Chill Neutralization Solution
The Neutralization Solution (N 7411) can be stored at 2-8 °C since it should be chilled prior to use.

Procedure
All steps are carried out at room temperature.

1. Harvest Cells
   Pellet **150 ml** of an overnight culture by centrifugation at 5,000 x *g* for 10 minutes and discard the supernatant.

Note: The optimal volume of culture to use depends upon the strain, the plasmid, and the density of the culture. For best results, measure the absorbance of the overnight culture at 600 nm (A_{600}) and calculate the cell mass, where cell mass equals A_{600} x culture volume (ml). 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 calculated using the formula below:

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

2. Resuspend Cells
   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.

3. Lyse Cells
   Lyse the resuspended cells by adding **12 ml** of Lysis Solution. Immediately mix the contents by gently inverting 6 to 8 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 to 5 minutes until it becomes clear and viscous. **Do not allow lysis to proceed longer than 5 minutes**. Prolonged

Spin Format Protocol Modification

alkaline lysis may permanently denature the supercoiled plasmid DNA and may render it unsuitable for use in downstream applications.

4. Neutralize Lysate
   Confirm that Neutralization Solution is chilled to 2-8 ℃.

   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. Allow the lysate to incubate for 5 minutes. A white aggregate (cell debris, proteins, lipids, SDS, and chromosomal DNA) will form.

5. Spin Lysate
   Invert the tube 4-6 times. Pellet the cell debris by centrifuging at ³15,000 X g for 10-15 minutes. Cell debris, proteins, lipids, SDS, and chromosomal DNA should fall out of solution as a cloudy, viscous precipitate. Decant the supernatant into a clean 50ml Collection Tube.

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

7. DNA Binding Column Preparation
   Insert a GenElute Maxiprep Binding Column into a 50 ml collection tube. Add 12 ml of Column Preparation Solution to each column and centrifuge in a swinging bucket rotor at 3,000-5,000 X g for 1-2 minutes. Discard the eluate.

9. Bind Plasmid DNA
   Transfer the cleared lysate from step 6 to the prepared DNA Binding Column seated in a 50 ml collection tube and spin in a swinging bucket rotor at 3,000 x *g* for 2 minutes. Discard the eluate. Add the rest of the cleared lysate to the column and repeat the spin. Discard the eluate.

Important: The Binding Column will not accommodate the entire volume of lysate, so be careful not to overfill the column but **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.

10. Apply Wash Solution 1
    Add **12 ml** of Wash Solution 1 to the binding column and spin in a swinging bucket rotor at 3,000 x *g* for 2 minutes. Discard the eluate.

11. Apply Wash Solution 2
    Prior to first time use, be sure to prepare Wash Solution 2 by adding the appropriate volume of ethanol. Add **12 ml** of Wash Solution 2 to the binding column and spin in a swinging bucket rotor at 3,000 x *g* for 5 minutes.

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 3,000 x *g* for 5 minutes.

For maximum concentration of plasmid: Centrifuge the column/collection tube unit in a swinging bucket rotor at 1,000 x *g* for 5 minutes.

Elution Options

Centrifugation Speed	Typical Volume Recovered	Relative Yield	Relative Concentration
3,000 x *g*	2.5 ml	100%	100%
1,000 x *g*	1.2 ml	80%	175%

Spin Format Protocol Modification

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
Alcohol precipitation is only necessary if a more concentrated plasmid preparation is desired.

Transfer the eluate to a pyrogen-free (endotoxin-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 with silica-based systems and will have no effect on downstream applications. 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).

KV,PHC 04/16-1

Spin Format Protocol Modification

Troubleshooting Guide

Problem	Cause	Solution
Lysate is not clear after filtration; binding column becomes clogged	Cells have been stored improperly before use	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	The Binding Column was allowed to empty before all the lysate was loaded onto the column	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	Binding Column was not dried sufficiently after the second wash step	Increase the drying time of the column to 20 minutes following the second wash.
Poor or no plasmid DNA recovery	Cells overgrown or undergrown	Confirm cell density by taking absorbance at 600 nm. See Step 1, Preparation Instructions.
	Too many or few cells harvested	Confirm that an appropriate cell mass was used. See Step 1, under Procedure.
	Starting culture is too old	Streak a fresh plate from a freezer stock. Pick a single colony and prepare a new culture.
	Plasmid replication is poor	Confirm that the cells were grown in the appropriate medium with a selective antibiotic under optimized conditions.
	Antibiotic activity is insufficient	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.
	Wash Solution 2 is too concentrated	Confirm that Wash Solution 2 was diluted with the specified volume of ethanol. Keep the bottle tightly capped between uses to prevent evaporation.
	Alkaline lysis exceeded 5 minutes	Prolonged alkaline lysis may permanently denature plasmid DNA. Do not allow lysis to exceed 5 minutes.
	Precipitation of cell debris is incomplete	Thoroughly mix the lysate following the addition of the chilled Neutralization Solution.
	Lysis is incomplete	Too many cells harvested. See Step 1 under Procedure. Lyse cells 3 to 5 minutes until the mixture becomes clear and viscous.
Absorbance readings do not accurately reflect the quantity of plasmid	The plasmid DNA is contaminated with RNA; RNase A treatment is insufficient	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.
	The plasmid DNA is contaminated with chromosomal DNA	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	The background reading is high due to silica fines	Subtract background at A_{320} as described under DNA Quantitation. To remove silica fines, spin the eluted sample at 5,000 x *g* for 5 minutes and transfer the supernatant to a pyrogen-free (endotoxin-free) tube.
	Wash Solution 2 is diluted with ethanol containing impurities	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.

Spin Format Protocol Modification

Troubleshooting Guide (continued)

Problem	Cause	Solution
Additional band migrates behind supercoiled plasmid during electrophoresis	Some of the supercoiled plasmid DNA has become nicked	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	Some of the supercoiled plasmid DNA has become permanently denatured	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	Purification is incomplete	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.
	The plasmid DNA is permanently denatured	Do not allow the lysis reaction to proceed longer than 5 minutes. Prolonged alkaline lysis may permanently denature plasmid DNA.
	DNA concentration is too low	Precipitate the DNA and resuspend in a desired volume as described under DNA Concentration.
	Ethanol is present in the final elution	Increase the drying time of the column to 20 minutes following the second wash.
	High salt concentration in final elution	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 Code	Related Products	Product Code
Water, Molecular Biology Reagent	W 4502	DirectLoad™ Wide Range DNA Marker	D 7058
Ethidium bromide, aqueous, 10 mg/ml	E 1510	Escort II Transfection Reagent	L 6037
LB Broth, Sterile Liquid Media	L 2542	Escort V Kit-Enhanced	E 1029
LB Broth, EZMix™	L 7658	GenElute™ HP Plasmid Maxiprep Kits	NA0300-S NA0300 NA0310
LB Agar, EZMix™	L 7533		
Precast Agarose Gels, 1.0%, 8 well	P 5472		
TBE Buffer (10x Concentrate)	T 4415	GenElute™ HP Plasmid Midiprep Kits	NA0200-S NA0200
Gel Loading Solution	G 2526		

Spin Format Protocol Modification

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 3,000 x *g*	RPM at 5,000 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					
MP4(R)	215	SB	13.0	4,537	5,857
	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.