Genesee Scientific
A Life Science Company

Product Manual

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Plasmid DNA Maxiprep Kit

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Introduction

The Gene Choice® Endo-Free Plasmid Midiprep kit and the Endo-Free Plasmid Maxiprep kit combine the power of high binding technology with an innovative Endotoxin Removal Technology (ER) to deliver high-quality plasmid DNA with low endotoxin levels for use in eukaryotic transfection and in vitro experiments.

Endotoxins are lipopolysaccharides (LPS), found in the outer cell membrane of gram-negative bacteria such as E. coli. One E. coli cell contains around 2 million LPS molecules, each having hydrophobic, hydrophilic, and charged regions. Bacteria release small quantities of endotoxins during growth, and large quantities at death. At the time of lysis during plasmid purification, endotoxins are shed into the lysate. The chemical and physical properties that endotoxin molecules possess lead to their co-purification with plasmid DNA by behaving similarly on the surface of silica and anion-exchange resins. The Gene Choice® Endo-Free Plasmid system uses a specially formulated buffer that prevents endotoxin molecules from binding to the surface of the purification matrix. In addition, the Endo-Free Plasmid Midiprep and Maxiprep kits include specialized filter cartridges that replace the centrifugation step following alkaline lysis, thereby reducing purification time.

For the best interpretation of results, it is crucial that the purified plasmid DNA be free of endotoxins. Endotoxin contamination lowers transfection efficiencies for endotoxin sensitive cell lines. For gene therapy, endotoxin contamination should be of major concern since endotoxins have the potential to cause fever, endotoxic shock syndrome, and interfere with in vitro transfection into immune cells.

Storage and Stability

- Kit components are guaranteed for at least 12 months from the date of purchase when stored as follows
  After RNase A is added, Solution 1 should be stored at 2-8˚C
- All other components should be stored at room temperature
- Store Solution 2 tightly capped when not in use
- During shipment or storage in cool ambient conditions, precipitates may form in GCE Buffer, Solution 2, and ER1 Binding Buffer. Dissolve such deposits by warming the solution at 37°C and gently shakin

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Yield and Quality of DNA

Determine the absorbance of an appropriate dilution (20 to 50-fold) of the sample at 260 nm and then at 280 nm.
The DNA concentration is calculated as follows:

$$\text{DNA Concentration} = \text{Absorbance } 260 \times 50 \times (\text{Dilution Factor}) \ \mu g/ml$$

A ratio greater than 1.8 indicates greater than 90% nucleic acid. Alternatively, quantity (as well as quality) can sometimes best be determined by agarose gel/ethidium bromide electrophoresis by comparison to DNA samples of known concentrations. Typically, the majority of the DNA eluted is in monomeric supercoil form, though concatamers may also be present.

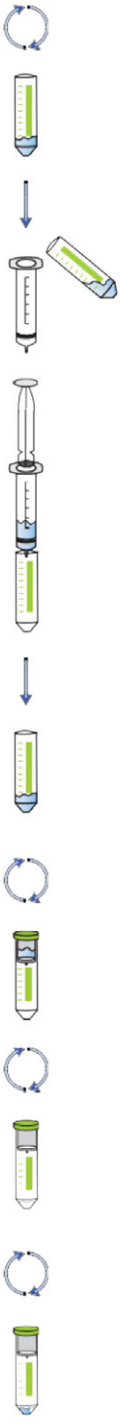
Plasmid Copy Number and Expected Yield

The yield and quality of the plasmid DNA obtained depends on a number of factors including plasmid copy number, size of insert, host strain, culture volume, culture medium, and binding capacity of the kits. Of these factors, the vector copy number, culture volume, and kit binding capacity are most important. Plasmid copy number ranges from one copy to several hundred copies per cell as dictated by their origin of replication. But very large plasmids often display a very low copy number per cell. The expected yield of 50 ml overnight cultures (LB medium) with the Gene Choice® Endo-Free Plasmid DNA Midi or Maxi Kit are indicated in the following table.

Sample Yields from 50 ml Starting Culture

Plasmid	Replicon	Copy Number	Expected Yield (50 ml culture)
pUC vectors	pMBI	500-700	150-250 µg
pBluescript® vectors	ColE14	300-500	100-180 µg
pGEM® vectors	pMB1	300-400	100-200 µg
pBR322 and its derivatives	pMB1	15-20	10-20 µg
ColE14	ColE14	15-20	10-20 µg
PACYC and its derivatives	p15A	37-40	5-10 µg
pSC101 and its derivatives	pSC101	~5	5 µg
pGEM	pMB1	300-700	100-200 µg

Centrifugation Protocol



Pellet by Centrifugation

Resuspend Lyse Neutralize

Transfer Lysate to Syringe

Insert Plunger Into Syringe and Clear Lysate into 15ml Collection Tube

Add ER1 Binding Buffer

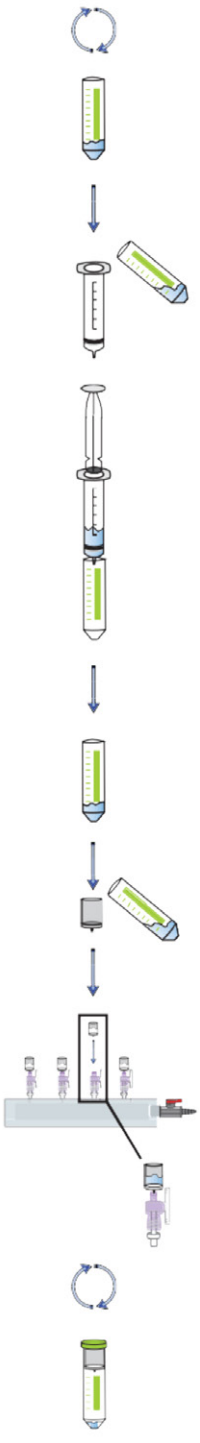
Pass Lysate through DNA Column

Wash 4X

Dry Matrix

Elute Endotoxin-free DNA

Vacuum Protocol



Pellet by Centrifugation

Resuspend Lyse Neutralize

Transfer Lysate to Syringe

Insert Plunger into Syringe and Clear Lysate into 15ml Collection Tube

Prepare Vacuum Manifold

Add ER1 Binding Buffer

Insert Column Into Leur Connector On Vacuum Manifold

Pass Lysate Through DNA Column

Wash 4X

Dry Matrix

Elute Endotoxin-free DNA

Kit Contents

Product	SMP96-555	96-555
Purifications	2	25
DNA Maxi Columns	2	25
50 mL Collection Tubes	2	25
Lysate Clearance Filter Syringe	2	25
Solution 1	30 ml	270 ml
Solution 2	30 ml	270 ml
Neutralization Buffer	15 ml	135 ml
ER1 Binding Buffer	55 ml	650 ml
ER2 Wash Buffer	25 ml	270 ml
GCE Buffer	14 ml	165 ml
DNA Wash Buffer	25 ml	200 ml
ETF Elution Buffer	30 ml	125 ml
RNase A	120 µl	1.2 ml
User Manual	✓	✓

Preparing Reagents

1. Add vial of RNase A to the bottle of Solution 1 provided and store at 2-8˚C

2. Dilute DNA Wash Buffer with 100% ethanol as follows and store at room temperature

Kit	100% Ethanol to be Added
SMP96-555	100 ml
96-555	800 ml

3. Dilute GCE Buffer with 100% isopropanol as follows and store at room temperature

Kit	100% Isopropanol to be Added
SMP96-555	9.5 ml
96-555	110 ml

4. Check Solution 2, GCE Buffer, and ER1 Binding Buffer for precipitates before use. Redissolve any precipitates by warming to 37˚C

Guidelines for Vacuum Manifold

The following is required for use with the Vacuum/Spin Protocol:

A) **Vacuum Manifold**
Compatible Vacuum Manifolds: Qiagen QIAvac24, Sigma AldrichVM20, Promega Vacman®, or manifold with standard Luer connector

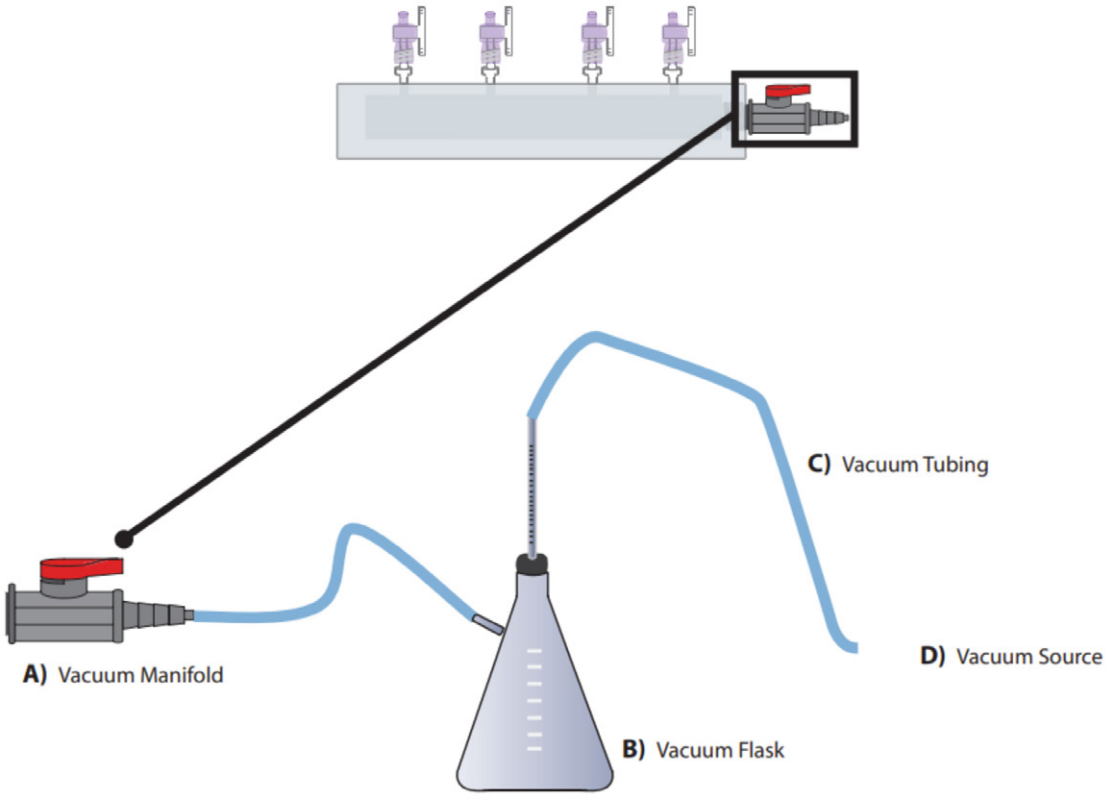
B) **Vacuum Flask**

C) **Vacuum Tubing**

D) **Vacuum Source** (review tables below for pressure settings)

Conversion from millibars:	Multiply by:
Millimeters of mercury (mmHg)	0.75
Kilopascals (kPa)	0.1
Inches of mercury (inch Hg)	0.0295
Torrs (Torr)	0.75
Atmospheres (atmos)	0.000987
Pounds per Square Inch (psi)	0.0145

Vacuum Setup:



Growth and Culture of Bacteria

Bacterial Strain Selection

It is strongly recommended that an end A negative strain of *E. coli* be used for routine plasmid isolation. Examples of such strains include DH5α™, DH1, and C_{600}. These host strains yield high-quality DNA with Gene Choice® Endo-Free Plasmid DNA kit protocols. XL1- Blue, although a slower growing strain is also recommended due to its yield of high- quality DNA.

Host strains derivatives from HB101 such as TG1 and the JM100 series release large amounts of carbohydrates during lysis, which may inhibit enzyme activity when not completely removed. Some strains may also lower DNA quality due to having high levels of endonuclease activity, and therefore are not recommended (i.e. JM101, JM110, HB101). One may reduce the amount of culture volume or double the volumes of Solution 1, Solution 2, and Neutralization Buffer, if problems are encountered with strains such as TG1 and Top10F.

Inoculation

Bacterial cultures for plasmid preparations should always be grown from a single colony picked from a freshly streaked plate. Subculturing directly from glycerol stock or liquid cultures may lead to uneven yields or plasmid loss. Optimal results are obtained by using one single isolated colony from a freshly transformed or freshly streaked plate to inoculate an appropriate volume of starter culture containing the appropriate antibiotic, and then incubated for 12-16 at 37°C with vigorous shaking (~300 rpm; shaking incubator).

Note: Aeration is especially important. The culture volume should not exceed 1/4 the volume of the container.

Culture Media

The Gene Choice® Endo-Free Plasmid DNA kits are specially designed for use with cultures grown in Luria Bertani (LB) medium. Richer broths such as TB (Terrific Broth) or 2 x YT lead to high cell densities that can overload the purification system, and therefore are not recommended. If rich media must be used, growth times must be optimized, and the recommended culture volumes must be reduced to match the capacity of the DNA Column.

Note: As culture ages, DNA yield may begin to decrease due to cell death and lysis within the culture.

Culture Volume and Cell Density

Do Not Exceed Maximum Recommended Culture Volumes

For optimal plasmid yields, the starting culture volume should be based on culture cell density. A bacterial density between 2.0 and 3.0 at OD_{600} is recommended. When using nutrient-rich media, care should be taken to ensure that the cell density does not exceed an OD_{600} of 3.0. Using a high-density culture outside of the recommended OD range may overload the purification system.

Vacuum Manifold Protocol

All centrifugation steps should be performed with a swing bucket rotor for maximum plasmid DNA yields. All centrifugation steps should be carried out at room temperature. If high yields of low copy number plasmid DNA are desired, see "Low Copy Number Plasmids and Cosmids" protocol.

Materials and Equipment to be Supplied by User:

- 100% ethanol (Do not use denatured alcohol)
- 100% isopropanol
- Centrifuge with swing bucket rotor capable of at least 4,000 x *g* (See the "Troubleshooting" section if not available)
- Nuclease-free 50 mL centrifuge tubes
- Appropriate centrifuge bottle for **Step 1**
- Vacuum manifold
- Vortexer
- Ice bucket
- **Optional:** Water bath or incubator capable of 70°C
- **Optional:** 3M NaOH

Before Starting:

- Chill Neutralization Buffer on ice
- Heat Elution Buffer to 70°C if Plasmid DNA is >10 kb
- Prepare DNA Wash Buffer, GCE Buffer, and Solution 1 according to "Preparing Reagents" section

1. Transfer 50-200 ml overnight culture to an appropriate centrifuge bottle (not provided).
Note: The optimal volume to use depends on the culture density and plasmid copy number. The optimal cell mass (OD_{600} x ml culture) for the DNA Maxi Column is 300-400. For example, if the OD_{600} of a culture is 4.0, the optimal culture volume should be 75-100 mL. If excess culture cell mass is used, alkaline lysis will be inefficient, the membrane will be overloaded, and the performance of the system will be decreased. The increase in lysate viscosity will require vigorous mixing which may result in shearing of genomic DNA and contamination the plasmid DNA. For low copy number plasmids, see the "Low Copy Number Plasmids" protocol.

2. Centrifuge at 4,000 x *g* for 10 minutes at room temperature

3. Decant or aspirate and discard the culture media
Note: To ensure that all traces of the medium are removed, use a clean paper towel to blot excess liquid from the wall of the bottle.

4. Add 10 ml Solution 1/RNase A. Vortex or pipet up and down to mix thoroughly. Complete resuspension of cell pellet is vital for obtaining good yields.
Note: RNase A must be added to Solution 1 before use. Please see the instructions in the "Preparing Reagents" section.

5. Add 10 ml Solution 2. Invert and rotate the tube gently 8-10 times to obtain a cleared lysate. This may require a 2-3 minute incubation at room temperature with occasional mixing.
Note: Avoid vigorous mixing as this will shear chromosomal DNA and lower plasmid purity. Do not allow the lysis reaction to proceed more than 5 minutes. Store Solution 2 tightly capped when not in use to avoid acidification from CO_2 in the air.

6. Add 5 ml cold Neutralization Buffer. Gently invert 10 times or until a flocculent white precipitate forms. This may require a 2-minute incubation at room temperature with occasional mixing
Note: The solution must be mixed thoroughly. This is vital for obtaining good yields. If the mixture still appears viscous, brownish, or conglobated, more mixing is required to completely neutralize the solution.

7. Prepare a Lysate Clearance Filter Syringe by removing the plunger. Place the barrel in a tube rack to keep upright. Make sure the end cap is attached to the syringe tip

8. Immediately transfer the lysate from **Step 6** into the barrel of the Lysate Clearance Filter Syringe

9. Hold the Lysate Clearance Filter Syringe barrel over a new 50 ml centrifuge tube (not provided) and remove the end cap from the syringe tip

10. Gently insert the plunger into the barrel to expel the cleared lysate into the 50 mL centrifuge tube
Note: Some of the lysate may remain in the flocculent precipitate. DO NOT force this residual lysate through the filter.

11. Measure the volume of cleared lysate

12. Add 1 volume ER1 Binding Buffer. Invert the tube gently 10 times

13. Prepare the vacuum manifold by following the manufacturer's instructions

14. Connect the DNA Maxi Column to the vacuum manifold. Refer to the **Illustrated Vacuum Set Up** for details

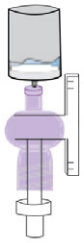
Optional Protocol for Column Equilibration:

1. Add 3 ml 3M NaOH to the DNA Midi Column
2. Let sit at room temperature for 5 minutes
3. Turn on the vacuum source to draw the buffer through the column
4. Turn off the vacuum

15. Transfer the cleared lysate from **Step 12** to the DNA Maxi Column

16. Turn on the vacuum source to draw the lysate through the column

17. **Continue adding lysate into until you have ~500 µl lysate remaining in the column**
Note: Leaving the ~500 µl lysate in the column prevents foaming in the following steps and allows for faster processing. See the illustration below.



Leaving 500 µl Solution 1n the column **after Step 21** reduces foam which decreases total processing time and improves efficiency.

18. Turn off the vacuum

19. Add 10 ml ER2 Wash Buffer

20. Turn on the vacuum source to draw the buffer through the column

21. Turn off the vacuum

22. Add 10 ml GCE Buffer
Note: GCE Buffer must be diluted with 100% isopropanol prior to use. Please see the instructions in the "Preparing Reagents" section.

23. Turn on the vacuum source to draw the buffer through the column

24. Turn off the vacuum

25. Add 15 ml DNA Wash Buffer
Note: DNA Wash Buffer must be diluted with 100% ethanol prior to use. Please see the instructions in the "Preparing Reagents" section.

26. Turn on the vacuum source to draw the buffer through the column

27. Turn off the vacuum

28. Add 10 ml DNA Wash Buffer.

29. Turn on the vacuum source to draw the buffer through the column

30. Turn off the vacuum

31. Transfer the DNA Maxi Column to a 50 ml Collection Tube (provided)

32. Centrifuge the empty DNA Maxi Column at 4,000 x *g* for 10 minutes to dry the column matrix
Note: It is important to dry the DNA Maxi Column matrix before elution. Residual ethanol may interfere with downstream applications.

33. Transfer the DNA Maxi Column to a nuclease-free 50 ml centrifuge tube

34. Add 1.5-3 ml ETF Elution Buffer directly to the center of the column matrix

35. Let it sit at room temperature for 5 minutes

36. Centrifuge at 4,000 x *g* for 5 minutes
Note: This represents approximately 65-80% of bound DNA. An optional second elution will yield any residual DNA, though at a lower concentration. Alternatively, a second elution may be performed using the first eluate to maintain a high DNA concentration.

37. Store DNA at -20°C

Notes

Centrifugation Protocol

The following protocol is designed for plasmid DNA isolation when a vacuum manifold is not available. All centrifugation steps should be performed with a swing bucket rotor for maximum plasmid DNA yields. All centrifugation steps should be carried out at room temperature. If high yields of low copy number plasmid DNA are desired, see "Low Copy Number Plasmids and Cosmids" protocol.

Materials and Equipment to be Supplied by User:

- 100% ethanol (Do not use denatured alcohol)
- 100% isopropanol
- Centrifuge with swing bucket rotor capable of at least 4,000 x *g* (See the "Troubleshooting" section if not available)
- Vortexer
- Nuclease-free 50 ml centrifuge tubes
- Appropriate centrifuge bottle for **Step 1**
- Ice bucket
- **Optional:** Water bath or incubator capable of 70°C
- **Optional:** 3M NaOH

Before Starting:

- Chill Neutralization Buffer on ice
- Heat Elution Buffer to 70°C if Plasmid DNA is >10 kb
- Prepare DNA Wash Buffer, GCE Buffer, and Solution 1 according to "Preparing Reagents" section

1. Transfer 50-200 ml overnight culture to an appropriate centrifuge bottle

Note: The optimal volume to use depends on the culture density and plasmid copy number. The optimal cell mass (OD_{600} x ml culture) for the DNA Maxi Column is 300-400. For example, if the OD_{600} of a culture is 4.0, the optimal culture volume should be 75-100 ml. If excess culture cell mass is used, alkaline lysis will be inefficient, the membrane will be overloaded, and the performance of the system will be decreased. The increase in lysate viscosity will require vigorous mixing which may result in shearing of genomic DNA and contamination the plasmid DNA. For low copy number plasmids, see the "Low Copy Number Plasmids" protocol.

2. Centrifuge at 4,000 x *g* for 10 minutes at room temperature.

3. Decant or aspirate and discard the culture media

Note: To ensure that all traces of the medium are removed, use a clean paper towel to blot excess liquid from the wall of the bottle

4. Add 10 ml Solution 1/RNase A. Vortex or pipet up and down to mix thoroughly. Complete resuspension of cell pellet is vital for obtaining good yields

Note: RNase A must be added to Solution 1 before use. Please see the instructions in the "Preparing Reagents" section.

5. Add 10 ml Solution 2. Invert and rotate the tube gently 8-10 times to obtain a cleared lysate. This may require a 2-3 minute incubation at room temperature with occasional mixing

Note: Avoid vigorous mixing as this will shear chromosomal DNA and lower plasmid purity. Do not allow the lysis reaction to proceed more than 5 minutes. Store Solution 2 tightly capped when not in use to avoid acidification from CO_2 in the air.

6. Addd 5 ml cold Neutralization Buffer. Gently invert 10 times or until a flocculent white precipitate forms. This may require a 2-minute incubation at room temperature with occasional mixing
Note: The solution must be mixed thoroughly. This is vital for obtaining good yields. If the mixture still appears viscous, brownish, or conglobated, more mixing is required to completely neutralize the solution.

7. Prepare a Lysate Clearance Filter Syringe by removing the plunger. Place the barrel in a tube rack to keep upright. Make sure the end cap is attached to the syringe tip

8. Immediately transfer the lysate from **Step 6** into the barrel of the Lysate Clearance Filter Syringe

9. Hold the Lysate Clearance Filter Syringe barrel over a new 50 ml centrifuge tube (not provided) and remove the end cap from the syringe tip

10. Gently insert the plunger into the barrel to expel the cleared lysate into the 50 ml centrifuge tube
Note: Some of the lysate may remain in the flocculent precipitate. DO NOT force this residual lysate through the filter.

11. Measure the volume of cleared lysate

12. Add 1 volume ER1 Binding Buffer. Invert the tube gently 10 times
Note: Steps 13-35 should be performed in a swing bucket rotor for maximum plasmid DNA yield. All of centrifugation steps should be carried out at room temperature.

13. Insert a DNA Maxi Column into a 50 ml Collection Tube (provided)

Optional Protocol for Column Equilibration:

1. Add 3 ml 3M NaOH to the DNA Maxi Column
2. Let sit at room temperature for 4 minutes
3. Centrifuge at 4,000 x *g* for 3 minutes
4. Discard the filtrate and reuse the collection tube

14. Transfer 20 ml cleared supernatant from **Step 12** to the DNA Maxi Column

15. Centrifuge at 4,000 x *g* for 3 minutes

16. Discard the filtrate and reuse the collection tube

17. Repeat **Steps 14-16** until all of the cleared supernatant has been transferred to the DNA Maxi Column

18. Add 10 ml ER2 Wash Buffer

19. Centrifuge at 4,000 x *g* for 3 minutes

20. Discard the filtrate and reuse the collection tube

21. Add 10 ml GCE Buffer
Note: GCE Buffer must be diluted with 100% isopropanol prior to use. Please see the instructions in the "Preparing Reagents" section.

22. Centrifuge at 4,000 x *g* for 3 minutes

23. Discard the filtrate and reuse the collection tube

24. Add 15 ml DNA Wash Buffer
Note: DNA Wash Buffer must be diluted with 100% ethanol prior to use. Please see the instructions in the "Preparing Reagents" section.

25. Centrifuge at 4,000 x *g* for 3 minutes

26. Discard the filtrate and reuse the collection tube

27. Add 10 ml DNA Wash Buffer

28. Centrifuge at 4,000 x *g* for 3 minutes

29. Discard the filtrate and reuse the collection tube

30. Centrifuge the empty DNA Maxi Column at 4,000 x *g* for 10 minutes to dry the column matrix
Note: It is important to dry the DNA Maxi Column matrix before elution. Residual ethanol may interfere with downstream applications.

31. Transfer the DNA Maxi Column to a nuclease-free 50 ml centrifuge tube

32. Add 1.5-3 ml ETF Elution Buffer directly to the center of the column matrix

33. Let it sit at room temperature for 5 minutes

34. Centrifuge at 4,000 x *g* for 5 minutes
Note: This represents approximately 65-80% of bound DNA. An optional second elution will yield any residual DNA, though at a lower concentration. Alternatively, a second elution may be performed using the first eluate to maintain a high DNA concentration.

35. Store DNA at -20°C

DNA Precipitation

The concentration of the eluted plasmid DNA varies with copy number, host strain, and growth conditions. In some cases, residual ethanol may also be present. To adjust the DNA concentration following plasmid DNA elution or for the removal of residual ethanol, perform the following isopropanol precipitation protocol.

1. Carefully transfer the eluted plasmid DNA to a clean tube suitable for precipitation. Add 1/10 volume 3M NaAC (pH 5.2) and 0.7 volume 100% isopropanol (room temperature). Vortex to mix

2. Centrifuge at ≥15,000 x *g* for 20 minutes at 4ºC

3. Carefully decant the supernatant

4. Add 1-2 ml 70% ethanol. Vortex to resuspend the pellet

5. Centrifuge at ≥15,000 x *g* for 10 minutes at 4ºC

6. Carefully decant the supernatant

7. Air dry the pellet for 10 minutes

8. Add 200-500 µl ETF Elution Buffer

9. Store DNA at -20°C

Low Copy Number Plasmid and Cosmid DNA Protocol

Low copy number plasmids generally give 0.1-1 µg DNA per ml overnight culture. For the isolation of plasmid DNA from low copy number plasmids (0.1-1 µg/ml culture) or low copy number plasmid (1-2 µg/ml culture) bacteria, use the following modified protocol.
Note: The Gene Choice®Endo-Free Plasmid DNA Midi kit and the Endo-Free Plasmid DNA Maxi kit come with enough buffers to perform the standard protocols. Additional buffers are needed to perform the Low Copy Number Plasmid and Cosmid DNA Protocol. These buffers can be purchased separately.

1. Increase the volume of starting culture from that of high copy number plasmids. Use 50-100 ml bacterial culture for the Gene Choice® Endo-Free Plasmid DNA Midi Kit and 200-400 ml bacterial culture for the Endo-Free Plasmid DNA Maxi Kit
2. Pellet the bacterial cells by centrifugation
3. Decant or aspirate and discard the culture media
4. Perform **Steps 4-12** in the standard protocols with double volumes of Solution 1, Solution 2, Neutralization Buffer, and ER1 Binding Buffer
5. Continue with **Step 13** of the standard protocols by following the wash, drying, and elution steps. ***There is no need to increase the volumes of GCE Buffer, ER2 Wash Buffer, DNA Wash Buffer, or ETF Elution Buffer***

Plasmid DNA Prepared by Other Methods Protocol

Plasmid DNA isolated using other methods can be further purified with the following protocol. The protocol includes the removal of endotoxins from the sample.

1. Bring the volume of plasmid DNA to 2 ml with sterile deionized water
2. Add an equal volume ER1 Binding Buffer. Vortex to mix thoroughly
3. Transfer the sample to the DNA Column. Draw the sample through the membrane by centrifugation or vacuum
4. Continue the appropriate protocol beginning with the DNA Wash Buffer step and finishing with the elution step

Troubleshooting Guide

Please use this guide to troubleshoot any problems that may arise. For further assistance, **please contact the technical support staff**.

Possible Problems and Suggestions

Low DNA Yields

Poor cell lysis	• Reduce the initial volume of culture or increase the lysis time while monitoring the lysis visually • Cells may not have been dispersed adequately prior to the addition of Solution 2. Make sure to vortex cell suspension to completely disperse • Solution 2, if not tightly closed, may need to be replaced
Bacterial culture is overgrown or not fresh	• Do not incubate cultures for more than 16 hours at 37ºC • Storage of cultures for extended periods prior to plasmid isolation is detrimental
Low elution efficiency	• If using endotoxin-free water for elution, the pH must be 8.0
Low copy number plasmid used	• Such plasmids may yield as little as 0.1 µg plasmid DNA from a 1ml overnight culture • Double culture volume and follow the Low Copy Number Plasmid and Cosmid DNA Protocol
Columns were spun in a fixed angle rotor or with insufficient g-force	• For the Midi and Maxi kits, the columns must be spun in a swing bucket rotor at 4,000 x *g* for liquids to pass through efficiently
Alkaline lysis is prolonged	• Reduce the lysis time (Solution 2) to 3 minutes or until the suspended cells form a clear viscous solution
Too many or too few cells were used	• Confirm the cell density by measuring OD. To calculate the volume of culture to use, take the desired cell mass and divide by the absorbance of the overnight culture at 600nm

No DNA Eluted

DNA Wash Buffer not diluted with ethanol	• Prepare DNA Wash Buffer according to the instructions
GCE Buffer not diluted with isopropanol	• Prepare GCE Buffer according to the instructions

High molecular weight DNA contamination of product

Over mixing of cell lysate upon addition of Solution 2	• Do not vortex or mix aggressively after adding Solution 2
Culture overgrown	• Overgrown culture contains lysed cells and degraded DNA. Do not grow cell longer than 16 hours

Plasmid DNA floats out of well while loading agarose gel

Ethanol has not been removed completely from column following wash steps	• Centrifuge column as instructed to dry the column before elution • Incubate columns for 10 minutes at 65°C to completely dry membrane after centrifugation step

Absorbance of purified DNA does not accurately reflect quality of the plasmid (A260/A280 ratio is too high or too low)

DNA Wash Buffer is diluted with ethanol containing impurities	• Check the absorbance of the ethanol between 250 nm and 300 nm. Do not use ethanol with high absorbance • Trace impurities may remain on the column after washing and can contribute to the absorbance
Plasmid DNA is contaminated with RNA; RNase A treatment is insufficient	• Confirm that the RNase A was added to Solution 1 prior to first use • The RNase A Solution may degrade due to high temperatures (>65°C) or prolonged storage (>6 months at room temperature)
Background reading is high due to fine silica particulates	• Spin the DNA sample at maximum speed for 1 minute; use the supernatant to repeat the absorbance readings
Purification is incomplete due to column overloading	• Reduce the initial volume of culture
Purification is incomplete due to column overloading	• Reduce the initial volume of culture
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 the lysis reaction or after adding Neutralization Buffer

4,000 x *g* centrifuge not available

For centrifuges only capable of 2,000-4,000 x *g*, increase all centrifugation times by 2 minutes except for the drying of the column. Increase drying by 5 minutes. It may be necessary to incubate the empty column for drying step at 65°C for 10 minutes to completely dry the column.

A Swing Bucket Centrifuge is Required

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