

Product Manual



Plasmid DNA Midiprep 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 shaking







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:**

DNA Concentration = Absorbance $260 \times 50 \times$ (Dilution Factor) µg/ml

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	рМВІ	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
*****			• • • • • • • • • • • • • • • • • • • •









Kit Contents

roduct	SMP96-550	96-550
urifications	2	25
NA Midi Columns	2	25
5 ml Collection Tubes	2	25
sate Clearance Filter Syringe	2	25
olution 1	6 ml	80 ml
lution 2	6 ml	80 ml
utralization Buffer	3 ml	40 ml
1 Binding Buffer	15 ml	170 ml
2 Wash Buffer	6 ml	60 ml
E Buffer	4 ml	48 ml
IA Wash Buffer	5 ml	50 ml
F Elution Buffer	15 ml	60 ml
lase A	20 µl	300 µl
ser 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

100% Ethanol to be Added
20 ml
200 ml

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

Kit	100% Isopropanol to be Added	
SMP96-550	2.6 ml	
96-550	32 ml	

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





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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)

Multiply by:
0.75
0.1
0.0295
0.75
0.000987
0.0145









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 C600. 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 OD₆₀₀ is recommended. When using nutrient-rich media, care should be taken to ensure that the cell density does not exceed an OD₆₀₀ of 3.0. Using a high-density culture outside of the recommended OD range may overload the purification system.





Endo-Free Plasmid DNA Midi

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 15 ml and 50 ml centrifuge tubes
- 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 20-50 ml overnight culture to a 50 ml centrifuge tube (not provided)

Note: The optimal volume to use depends on the culture density and plasmid copy number. The optimal cell mass (OD₆₀₀ x ml culture) for the DNA Midi Column is 80-100. For example, if the OD₆₀₀ of a culture is 4.0, the optimal culture volume should be 20-25 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 tube

4. Add 2.5 ml Solution 1/RNase A. Vortex or pipet up and down to completely resuspend the cells Note: RNase A must be added to Solution 1 before use. Please see the instructions in the "Preparing Reagents" section

5. Add 2.5 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 CO₂ in the air.





6. Add 1.25 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 clean 15 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 15 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 Midi Column to the vacuum manifold. Refer to the Illustrated Vacuum Set Up for details

Optional Protocol for Column Equilibration:

Add 1 ml 3M NaOH to the DNA Midi Column

- Turn on the vacuum source to draw the buffer through the column
- Turn off the vacuum
- 15. Transfer the cleared lysate from Step 13 to the DNA Midi 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 2 ml ER2 Wash Buffer

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

21. Turn off the vacuum

22. Add 3 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 3.5 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. Repeat Steps 25-27 for a second DNA Wash Buffer wash step
- 29. Transfer the DNA Midi Column to a 15 ml Collection Tube (provided).

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

- 31. Transfer the DNA Midi Column to a nuclease-free 15 ml centrifuge tube
- 32. Add 0.5-1 ml ETF Elution Buffer directly to the center of the column matrix
- 33. Let it sit at room temperature for 3 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





Endo-Free Plasmid DNA Midi

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)
- Nuclease-free 15 ml and 50 ml centrifuge tubes
- 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 20-50 ml overnight culture to a 50 ml centrifuge tube (not provided)

Note: The optimal volume to use depends on the culture density and plasmid copy number. The optimal cell mass (OD₆₀₀ x ml culture) for the DNA Midi Column is 80-100. For example, if the OD₆₀₀ of a culture is 4.0, the optimal culture volume should be 20-25 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 tube.

4. Add 2.5 ml Solution 1/RNase A. Vortex or pipet up and down to completely resuspend the cells Note: RNase A must be added to Solution 1 before use. Please see the instructions in the "Preparing Reagents" section.

5. Add 2.5ml 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 CO2 in the air.





6. Add 1.25 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 clean 15 ml centrifuge tube (not provided) and remove the end cap from the syringe tip

Gently insert the plunger into the barrel to expel the cleared lysate into the 15 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 13-32 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 Midi Column into a 15 ml Collection Tube (provided)

Optional Protocol for Column Equilibration:

- Add 1 ml 3M NaOH to the DNA Midi Column
- Let sit at room tem perature for 4 minutes
- Centrifuge at 4,000 x g for 3 minutes
- Discard the filtrate and reuse the collection tube

14. Transfer 3.5 ml cleared supernatant from Step 12 to the DNA Midi 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 Midi Column
- 18. Add 2 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 3 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 3.5 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. Repeat Steps 24-26 for a second DNA Wash Buffer wash step

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

- 29. Transfer the DNA Midi Column to a nuclease-free 15 ml centrifuge tube
- 30. Add 0.5-1 ml ETF Elution Buffer directly to the center of the column matrix
- 31. Let it sit at room temperature for 3 minutes

32. 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.

33. 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





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 37°C 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 <i>g</i> 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 w	rhile 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 doe	es not accurately reflect quality of the plasmid (A ₂₆₀ /A ₂₈₀ 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
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









