Endo-free Plasmid Midiprep Kit

E665631

Storage: All components of kit are stored at room temperature (15-30℃) for one year, it is recommended to store columns at 2-8℃ for longer periods. Following the addition of RNase A Solution, the Buffer P1 should be stored at 2-8℃ and is stable for 6 months.
Shipment: All components of the kit are shipped at room temperature.

Introduction:
Use the Endo-free Plasmid Midiprep Kits to isolate high yields of highly pure plasmid DNA. The kit utilizes silica-based membrane technology in the form of convenient spin columns. The adsorbed DNA is washed to remove contaminants and eluted with the Elution Buffer. The kit is designed to reduce low endotoxin level to plasmid DNA, and each prep recovers up to 100μg of high copy plasmid DNA that can be used in a wide variety of molecular biology procedures such as restriction endonuclease digestion, ligation, transformation, automated sequencing, in vitro transcription and transfection of sensitive cells.

Contents:

Endo-Free Plasmid Midiprep Kit			
Cat No.	Components	Size(50preps)	Storage
E665631A	Buffer P1	30 mL	RT
E665631B	Buffer P2	30 mL	RT
E665631C	Buffer E3	30 mL	RT
E665631D	Buffer PS	15 mL	RT
E665631E	Buffer PW (concentrated)	10 mL	RT
E665631F	Endo-free Buffer EB	10 mL	RT
E665631G	RNase A (10 mg/mL)	600μL	RT
E665631H	Buffer ER	8 mL	RT
E665631I	CWBlue	300μL	RT
E665631J	Spin Columns DL with Collection Tubes	50 EA	RT
E665631K	Endo-Remover FM with Collection	50 EA	RT

Additional materials: Ethanol (96-100%), Isopropanol.

Notes:
1. Add the RNase A Solution provided in the kit to the Buffer P1 and mix thoroughly. After the addition of RNase A, the Buffer P1 is stable for 6 months when stored at 4°C. Returning to room temperature before use.
2. Add the indicated volume of ethanol (96-100%) to Buffer PW (concentrated) prior to the first use.

3. Check the Buffer P2、Buffer E3、Buffer ER for salt precipitation before each use. Re-dissolve any precipitate by warming the solution to 37°C, then cool back down to 25°C. Do not vigorously shake the Buffer P2 Solution.

4. Wear gloves when handling the Buffer P2, Buffer E3 and Buffer PS bottles as these solutions contain irritants and are harmful if they come in contact with skin, or if they are inhaled or swallowed.

5. The spin column treated with Buffer PS should be left for 15-30 minutes before use, and it is efficient. It is not recommended to use it for more than 30 minutes.

6. The CWBlue contains volatile substances, and the lid should be immediately closed after use.

7. CWBlue is an indicator. Add the indicated volume of CWBlue: Buffer P1=1:100 and mix thoroughly. After adding Buffer P2 and mixing, if the solution shows a uniform and clear blue color, it shows that the bacterial cell lysis is complete. After adding Buffer E3 and mixing, the solution appears colorless and transparent, with white flocculent precipitates floating on it, showing that the neutralization reaction of refolding is complete.

Operation steps:

- **Protocol A. Plasmid DNA purification in quick version**

1. Grow up 5-15 mL of bacterial culture, harvest the cells by centrifugation for 1 min at 13,000 rpm(~16, 200×g). Discard the supernatant.

2. Resuspend pelleted cells in 500μL of Buffer P1. The bacterial pellet can be resuspended by vortexing or pipetting up and down until no cell clumps remain. Ensure that the RNase A Solution has been added to the Buffer P1 as described.

3. Add 500μL of Buffer P2 and mix gently by inverting the tube 8-10 times until the solution becomes viscous and slightly clear. Incubate for 5 min at room temperature.

Note:

1) Do not vortex to avoid shearing chromosomal DNA.
2) Do not incubate for more than 5 min to avoid denaturation of supercoiled plasmid DNA.
3) After adding Buffer P2, if the solution shows a uniform and clear blue color, it shows that the bacterial cell lysis is complete.

4. Add 200μL Buffer PS to the Spin Columns DL that has been pre-assembled with collection tube (provided), centrifuge at 13,000 rpm for 2 minutes, discard the flow-through and place the column into the same collection tube.

Note: When the column is balanced, it is recommended to stand it for 15-30 minutes until the 7th step is used (it is recommended not to exceed 30 minutes), which can improve the performance of the column and increase the extraction yield.

5. Add 500μL of the Buffer E3 and mix immediately by inverting the tube 8-10 times. Incubate 5 min at room temperature, centrifuge for 5 min at 13,000 rpm. Transfer the supernatant to an Endo-Remover FM Column pre-assembled with collection tube (provided) by decanting or pipetting.

Note:

1) After the addition of the Buffer E3 it is important to mix the contents of the tube gently, but thoroughly, to avoid localized precipitation of bacterial cell debris. The neutralized bacterial lysate should appear cloudy and contain white precipitate.

2) Do not overfill the column. Use recommended centrifugation speed. Discard the flow-through and place the column back into the same collection tube.

6. Add isopropanol to 0.3 times the volume of supernatant, inverting the tube.

7. Transfer part of the sample (~ 750μL) to the column is balanced.

8. Do not overfill the column. Centrifuge for 1 min at 13,000rpm in a swinging bucket rotor. Discard the flow-through and place the column back into the same collection tube.

9. Add 750μL of Buffer PW (with ethanol added as described) to the purification column. Centrifuge for 1 min at 13,000rpm in a swinging bucket rotor. Discard the flow-through and place the column back into the same collection tube.

10. Centrifuge for 1 min at 13,000rpm in a swinging bucket rotor to remove residual ethanol. Discard the collection tube containing the flow-through.

11. Transfer the column to a fresh endotoxin-free 50 mL collection tube (not provided). Add 100-200μL of Buffer EB to the center of the purification column membrane. Incubate for 2-5 min at room temperature and centrifuge for 2 min at 13,000rpm to elute plasmid DNA.

Note:

1) To increase the concentration of eluted DNA the volume of the Buffer EB can be reduced to 100μL. Be aware that lower volumes of Buffer EB will decrease the overall yield of eluted DNA.
2) When the plasmid copy number is low or>10 kb, preheating Buffer EB in water bath at 65-70 ℃ can increase efficiency.

- **Protocol B. Plasmid DNA purification for endotoxin removal version**

1. Grow up 5-15 mL of bacterial culture, harvest the cells by centrifugation for 1 min at 13,000 rpm(~16, 200×g). Discard the supernatant.

2. Resuspend pelleted cells in 500μL of Buffer P1. The bacterial pellet can be resuspended by vortexing or pipetting up and down until no cell clumps remain. Ensure that the RNase A Solution has been added to the Buffer P1 as described.

3. Add 500μL of Buffer P2 and mix gently by inverting the tube 8-10 times until the solution becomes viscous and slightly clear. Incubate for 5 min at room temperature.

Note:

4) Do not vortex to avoid shearing chromosomal DNA.
5) Do not incubate for more than 5 min to avoid denaturation of supercoiled plasmid DNA.
6) After adding Buffer P2, if the solution shows a uniform and clear blue color, it shows that the bacterial cell lysis is complete.

4. Add 500μL of the Buffer E3 and mix immediately by inverting the tube 8-10 times. Incubate 5 min at room temperature, centrifuge for 5 min at 13,000 rpm. Transfer the supernatant to an Endo-Remover FM Column pre-assembled with collection tube (provided) by decanting or pipetting.

Note:

1) After the addition of the Buffer E3 it is important to mix the contents of the tube gently, but thoroughly, to avoid localized precipitation of bacterial cell debris. The neutralized bacterial lysate should appear cloudy and contain white precipitate.
2) Do not overfill the column. Use recommended centrifugation speed. Discard the flow-through and

place the column back into the same collection tube.

5. Add Buffer ER to 0.1 times the volume of the filtrate by inverting, ice bath for 30 mins, and then water bath at 60 ℃ for 10 mins.

6. Add 200μL Buffer PS to the Spin Columns DL that has been pre-assembled with collection tube (provided), centrifuge at 13,000 rpm for 2 minutes, discard the flow-through and place the column into the same collection tube.

Note: When the column is balanced, it is recommended to stand it for 15-30 minutes until the 7th step is used (it is recommended not to exceed 30 minutes), which can improve the performance of the column and increase the extraction yield.

7. After finishing the water bath of step 5, centrifuge for 10 mins at 8,000 rpm. A yellow oil phase appears at the bottom of the tube. Transfer the supernatant to a clean centrifuge tube (not provided) and be careful not to suck up the yellow oil phase at the bottom.

8. Add isopropanol to 0.3 times the volume of supernatant, inverting the tube.

9. Transfer part of the sample (~ 750μL) to the column is balanced.

10. Do not overfill the column. Centrifuge for 1 min at 13,000rpm in a swinging bucket rotor. Discard the flow-through and place the column back into the same collection tube.

11. Add 750μL of Buffer PW (with ethanol added as described) to the purification column. Centrifuge for 1 min at 13,000rpm in a swinging bucket rotor. Discard the flow-through and place the column back into the same collection tube.

12. Centrifuge for 1 min at 13,000rpm in a swinging bucket rotor to remove residual ethanol. Discard the collection tube containing the flow-through.

13. Transfer the column to a fresh endotoxin-free 50 mL collection tube (not provided). Add 100-200μL of Buffer EB to the center of the purification column membrane. Incubate for 2-5 min at room temperature and centrifuge for 2 min at 13,000rpm to elute plasmid DNA.

Note:

3) To increase the concentration of eluted DNA the volume of the Buffer EB can be reduced to 100μL. Be aware that lower volumes of Buffer EB will decrease the overall yield of eluted DNA.
4) When the plasmid copy number is low or>10 kb, preheating Buffer EB in water bath at 65-70 ℃ can increase efficiency.

The output and quality of plasmid DNA depend on many factors, including plasmid copy number, insert fragment size, host strain, culture volume and so on. Copy numbers of various vectors make its output are also different. 5-15mL culture volume to use and output are shown in the following table:

Vectors	Type of vectors	Copy Numbers	Expected output
pUC vectors	High-copy	500-700 copies per cell	15-70μg
pBluescript vectors	High-copy	300-500 copies per cell	15-70μg
pGM vectors	High-copy	300-400 copies per cell	15-70μg
pBR322 and derivatives	Low-copy	15-20 copies per cell	5-25μg
PACYC and derivatives	Low-copy	37540 copies per cell	5-25μg