ApexPrep DNA Plasmid Miniprep Kit

Introductions

The ApexPrep DNA Plasmid Miniprep kit is based on alkaline lysis technology, which can lyse cells and then adsorb DNA to the adsorption membrane in the presence of high salt. Subsequent steps such as washing away impurities can efficiently and quickly extract up to 30 μg of plasmid DNA. This kit uses a unique buffer formula to maximize the removal of protein impurities and other organic compounds, and can process 1-5 ml of bacterial solution each time. The yield and quality of plasmid extraction are related to the type of host bacteria and culture conditions, cell lysis, plasmid copy number, plasmid stability, antibiotics, and other factors.

Plasmid DNA prepared by ApexPrep DNA Plasmid Miniprep Kit is suitable for a variety of routine applications including restriction enzyme digestion, sequencing, library screening, ligation and trans-formation, in vitro translation, and transfection of robust cells.

Materials

1. Components and storage

Components	50 tests	300 tests
Buffer BL	30 mL	160 mL
Buffer A1	20 mL	100 mL
Buffer A2	20 mL	100 mL
Buffer A3	30 mL	120 mL
Buffer AP	12 mL	50 mL
Buffer AE	15 mL	30 mL
RNase A	200 μL（10mg/mL）	1 mL（10mg/mL）
Spin Columns	50	300
Collection Tubes	50	300

Store at room temperature for one year. Buffer A1 with RNase A should be stored at 2-8°C.

2. Storage conditions

ApexPrep DNA Plasmid Miniprep Kit can be stored at room temperature for up to one year without showing any reduction in performance and quality. For longer storage, this kit can be stored at 2-8°C. RNase A can be stored for more than one year at room temperature. After addition of RNase A, Buffer A1 is stable for one year at 2-8°C.If any precipitate forms in the buffers after storage at 2-8°C, it should be dissolved by warming the buffers at 37°C before use.

3. Yield

Plasmid Type	Bacterial Cells Volume	Plasmid Yield	Plasmid
Low Copy	1-5 mL	3-12 μg	pBR322, pACYC, pSC101, SuperCos, pWE15
High Copy	1-5 mL	6-30 μg	pTZ, pUC, pBS, pGM-T

Protocols

1. Some preparations

(1) For the first use of the kit, please add 200 μL RNase A to buffer A1 before use, and then store buffer A1 at 2-8°C; add 48 mL of ethanol to buffer AP (50 tests) before use.

(2) For the first use of the kit, please add 1mL RNase A to buffer A1 before use, and then store buffer A1 at 2-8°C; add 200 mL of ethanol to buffer AP (300 tests) before use.

2. Procedure

(1) Column equilibration: place a spin column in a clean collection tube, and add 500 μL Buffer BL to spin column. Centrifuge for 1 min at 12,000 rpm (~13,400 × g) in a table-top microcentrifuge. Discard the flow-through, and put the spin column back into the collection tube. (Please use freshly treated spin column).

(2) Harvest 1-5 mL of the overnight cultured bacterial cells in a centrifuge tube by centrifugation at 12,000 rpm (~13,400×g) for 1 min to collect the bacterial pellet and try to remove the supernatant. (When there is a lot of bacterial liquid, the bacterial pellet can be collected into a centrifuge tube by multiple centrifugation).

(3) Add 250 μL Buffer A1 to the centrifuge tube, and resuspend the bacterial pellet.

(4) Add 250 μL of Buffer A2 to the centrifuge tube and gently invert 4-6 times to mix. When the bacteria are fully lysed, the bacteria liquid becomes clear and viscous.

Note: Mix gently. Do not shake vigorously. The time used in this step should not take more than 5 min to avoid damage to the plasmid.

(5) Add 350 μL of Buffer A3 to the centrifuge tube and immediately gently invert 4-6 times upside down to mix well. The solution should become cloudy.
Note: Buffer A3 should be mixed immediately after adding to avoid local precipitation.

(6) Centrifuge at 12,000 rpm (~13,400×g) for 10 min. Transfer the supernatant to the spin column that has been loaded into the collection tube. Be careful not to aspirate the precipitate. Centrifuge at 12,000 rpm (~13,400×g) for 30-60 sec. Discard the waste liquid in the collection tube and put the spin column into the collection tube.

(7) Add 750 μL Buffer AP to the spin column and centrifuge at 12,000 rpm (~13,400×g) for 30 sec. Discard the waste liquid in the collection tube and put the spin column back into the collection tube.

(8) Centrifuge the spin column with a collection tube at 12,000 rpm (~13,400×g) for 2 min to remove the remaining buffer in the spin column.

(9) Put the spin column into a clean centrifuge tube and add 30-50 μL of Buffer AE to the center of the spin column membrane. Incubate for 1 min.

(10) Centrifuge at 12,000 rpm (~13,400×g) for 2 min. Collect the plasmid solution in a centrifuge tube. Store the plasmid at -20°C.

Low copy or large plasmid (>10 kb) extraction

If the extracted plasmid is a low-copy plasmid or a large plasmid larger than 10 kb, the number of bacterial cells should be increased, such as harvesting 5-10 mL of overnight culture. At the same time, increase the amount of Buffer A1, A2, and A3 in proportion. Buffer AE should be preheated in a 65-70°C water bath, and the time can be extended appropriately during adsorption and elution to increase extraction efficiency. The other steps are the same.

Important Notes

(1) Check Buffer BL, Buffer A2 and Buffer A3 before use for salt precipitation. If necessary, dissolve the buffer by warming at 37°C for several minutes.

(2) Avoid direct contact of Buffer A2 and Buffer A3, immediately close the lid after use.

(3) All centrifugation steps are carried out at 12,000 rpm (~13,400× g) in table-tap microcentrifuge at room temperature.

(4) The amount of extracted plasmid is related to cells concentration and plasmid copy.

(5) Use Buffer BL to treat the spin column before the experiment to maximize the activation of the spin membrane and increase the yield.

(6) After treated with Buffer BL, use the spin column soon, otherwise long-term placement may affect the purifying effect.

APExBIO Technology
www.apexbt.com
7505 Fannin street, Suite 410, Houston, TX 77054.
Tel: +1-832-696-8203 | Fax: +1-832-641-3177 | Email: info@apexbt.com