For research purpose only. Not for use in diagnostic procedures for clinical purposes. For IN VITRO USE ONLY.

DNA-spin™ Plasmid DNA Purification Kit

Cat. No. 17093 250 columns

DESCRIPTION

DNA-spin™ Plasmid DNA Purification Kit provide a fast, efficient means of preparing high purity plasmid DNA without specialized devides or equipment. This kit contains a spin-type column filled with silica bead membrane and reagents optimizing alkali lysis for easy purification of plasmid DNA from bacteria. The specially treated silica bead membrane makes it easy to harvest high quality plasmid DNA by eliminating RNA and genomic DNA during the quick spin-down process. The plasmid DNA is free from protein, chromosomal DNA, RNA contaminants, and can be used directly in experiment.

STORAGE

Store at RT except for Resuspension buffer. Resuspension buffer shall be stored at 4 °C after adding RNase A solution.

KIT CONTENTS

- Resuspension buffer 65ml
  : Add RNase A solution 3.5ml to Resuspension buffer before use. Then, store at 4 °C.
- Lysis buffer 65ml
  : Check Lysis buffer for SDS precipitation due to low storage temperature, in which case it is necessary to dissolve the SDS by warming to 37 °C.
- Neutralization buffer 90ml
  : This buffer contains acetic acid.
- Washing buffer A 130ml
  : *end*A$^+$ strains such as HB101, the JM strains, NM series strains, PR series strains and some other wide-type strains have high endonucleases activity. endonucleases, that can degrade plasmid DNA are essentially removed by Washing buffer A of DNA-spin™ Kit.
- Washing buffer B 50ml
  : Add 200ml of absolute EtOH to the washing buffer before use.
- Elution buffer 20ml
  : DNase/RNase free Ultra-Pure solution.
- DNA-spin column (Yellow) 250 columns
  : Polypropylene tube containing silica membrane
- Collecting tube 250 tubes
  : Polypropylene tube for 2ml volume
- RNase solution 3.5ml
  : 10mg/ml concentration

ADDITIONAL REQUIRED EQUIPMENT

Absolute ethanol

Microcentrifuge tube (1.5ml, sterile) and standard microcentrifuge capable of a 10,000 X g.

CHARACTERISTICS

- Takes only 30 minutes to extract plasmid DNA.
- Highly purified plasmid DNA thanks to our specially treated plasmid DNA-specific silica bead membrane.
- Minimal nicks of plasmid DNA guarantees good results in plasmid DNA sequencing.

PROTOCOL

1. Pick a single colony from a freshly streaked bacterial plate and use it to inoculate LB plus an appropriate antiboitic. Incubate the culture overnight with shaking.
   Note : The optimal culture volume per one column is 3-5ml at OD_{600} of 1-1.5 (see the manual guide for DNA-spin Kit).
2. Harvest 3-5ml of bacterial culture by centrifugation at 13,000rpm for 30 sec at RT and discard the supernatant.
   Note : Remove all traces of supernatant by inverting the open centrifuge tube until all media has been drained. Relax the cell pellet by tapping the tip of tube.
3. Resuspend the pellet in 250μℓ of Resuspension buffer, vortexing or pipetting until no clumps of the cell pellet remain.
   Note : Ensure that RNase A Solution has been added to Resuspension buffer. It is essential to completely resuspend the cell pellet. It may affect the lysis efficiency.
4. Add 250μℓ of Lysis buffer to resuspended cells. Close tube and gently mix by inverting the tube several times. Do not vortex and do not exceed 5 min for lysis time.
   Note : The optimal lysis time allows maximum release of plasmid DNA without release of chromosomal DNA, while minimizing the exposure of the plasmid to denaturing conditions. Long exposure to alkaline condition a may cause the plasmid to become irreversibly denatured. Do not vortex, it may cause shearing of genomic DNA.
5. Add 350μℓ of Neutralization buffer and gently mix by inverting the tube several times.
   Note : Do not vortex. The solution should become cloudy and a flocculent precipitate should form. To improve the yield, place the tube at 4°C for 5min or use chilled Neutralization buffer to enhance the precipitation.
6. Centrifuge at 13,000rpm for 10 min at 4°C. While waiting for the centrifugation, insert a column into collection tube.
7. After centrifugation, transfer supernatant promptly into the column.
   Note : Cell debris, protein , and genomic DNA will form a compact white pellet in the tube. Do not transfer with white pellet.
8. Centrifuge at 13,000 rpm for 60 sec. Remove the column from collection tube, discard filtrate in collection tube. And then place the spin column back in the same collection tube.
9. **(Optional)** Add 500μℓ of Washing buffer A and centrifuge at 13,000rpm for 60 sec. Remove the column from collection tube, discard filtrate in collection tube. And then place the spin column back in the same collection tube.
   Note : This step is necessary to remove trace nuclease activity. *end*A$^+$ strains, such as HB101, JM series, or any wild-type strains, have high level of nuclease activity that can degrade plasmids. But *end*A$^-$ strains,such as DH5α, XL1-blue and etc, do not require this additional washing step.
10. Add 700μℓ of Washing buffer B, centrifuge at 13,000 rpm for 60 sec. Discard filtrate in the collection tube and place the spin column back in the same collection tube.
    Note : If the DNA will be used for salt sensitive applications, such as blunt-end ligation and direct sequencing, repeat the step10 using 500μℓ of Washing buffer B.
11. Centrifuge at 13,000 rpm for 60 sec to dry the filter membrane.
    Note : Completely remove ethanol.Residual ethanol from Washing buffer B may inhibit subsequent enzymatic reaction.
12. Put the column into a clean and sterile centrifuge tube. Add 50μℓ of Elution buffer or distilled water to the upper reservoir of the column, and let it stand for 1min. Then, centrifuge the tube assembly at 13,000 rpm for 60sec.
    Note : It is suggested to use at least 20μℓ of the Elution buffer to obtain best result. If the plasmid is low-copy-number or larger than 10Kb, the yield of plasmid may not be sufficient. In this case, pre-warmed (about 50°C) elution buffer will improve efficiency of elution.