



PRODUCT INFORMATION

EZ-500 SPIN COLUMN PLASMID DNA MAXI-PREP KIT

Kit Contents

Components	BS465, 10 Maxi-Preps	BS466, 20 Maxi-Preps
Solution I	245 ml	2x245 ml
Solution II	2x250 ml	4x250 ml
Solution III	4x225 ml	8x225 ml
Wash Solution	24 ml	48 ml
Elution Buffer	50 ml	100 ml
RNase A (10 mg/ml)	5 ml	10 ml
EZ-500 Column	10	20
50 ml Collection Tube	10	20
Protocol	1	1

- Solution II may form a precipitate upon storage. If necessary, dissolve the precipitate by warming at 37°C.
- Before use, add 96 ml of 100% ethanol to 24 ml Wash Solution for BS465, add 192 ml of 100% ethanol to 48ml Wash Solution for BS466. For other volumes of wash solution, simply add enough ethanol to make a 4:1 ratio (volume of added ethanol: volume of Wash Solution = 4:1).
- Elution Buffer is 2.0 mM Tris-HCl pH 8.0~8.5. Although TE buffer pH 8.0 or water can be used, yield is generally 20% lower.
- Before use, add RNase A to Solution I. Then Solution I should be stored at 4 °C for frequent use, or stored at -20 °C if not use for a long period.

Storage: With the exception of the RNase A, the kit may be stored at room temperature. The RNase A should be stored at 4°C. The kit is stable for 12 months at room temperature. For longer storage, keep all contents cold.

Principle:

This kit provides a simple and efficient method for purification of up to 500 ug of plasmid DNA. Bacterial cultures are lysed and the lysates are cleared by routine methods (Solution I, II, III). Plasmid DNA from the cleared lysate are selectively adsorbed in EZ-500 spin column and other impurities such as proteins, salts, oligos (<40-mer) and nucleotides are washed away. Pure DNA is eluted in low-salt buffer or water. Purified plasmid DNA can be used for any downstream applications such as sequencing, restriction reactions, labeling, transformation, PCR and Southern-blotting.

Features:

- Fast. Entire procedure takes 40-60 minutes.
- Preparation of high quality plasmid DNA from culture. Purified DNA can be used in any downstream applications such as sequencing, transformation, restriction enzymatic digestion, and transfections.
- High yields (80-90%) and Reproducible.
- No phenol / chloroform extraction; No CsCl centrifugation; No ethanol precipitation

Procedure for Purification of Plasmid DNA

1. Add 500 ml overnight culture to a appropriate centrifuge tube and centrifuge at 6,000 rpm for 10 minutes. Drain the liquid completely.



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2. Add 20 ml of Solution I to the pellet, mix gently and keep on ice for 2 minute.
3. Add 40 ml of Solution II to the mixture, mix gently by inverting the tube 4-6 times and then keep at RT for 2 minutes.

Note: To prevent contamination from genomic DNA, do not vortex.

4. Add 70 ml of Solution III, and mix gently. Incubate at RT for 2 minutes.
5. Spin at 10,000 rpm for 10 minutes.
6. Place column into a 50 ml collection tube. Transfer the above supernatant (step 5) to the column, Stand at RT for 5 minutes. Spin at 6000 rpm for 3-5 minutes.
7. Discard the flow-through in the tube. Add 5 ml of Wash Solution to the column, and spin at 8000 rpm for 3-5 minutes.
8. Repeat wash procedure in step 7
9. Discard the flow-through in the collection tube. Spin at 8000 rpm for additional 5 minutes to remove residual Wash Solution.
10. Transfer the column to a clean 50 ml microfuge tube. Add 2.5ml of Elution Buffer into the center part of the membrane of the column and incubate at 37 - 50°C for 2 minutes. Spin at 8000 rpm for 2 minutes. Store DNA at -20°C °C freezer. For higher recovery yield additional 2.5ml of Elution Buffer is added to the center part of the column and spin at 8000 rpm for 2 minutes. Measure OD260. Purified plasmid DNA is ready to use, or store at freezer for long period use.

Note: It is important to add the Elution Buffer into the center part. Pre-warm Elution Buffer at 55-80 °C. could increase elution efficiency. Two times elution is recommended.