



PRODUCT INFORMATION

EZ-200 SPIN COLUMN PLASMID DNA MEDI-PREPS KIT

Kit Contents

| Components | BS463, 10 Medi-Preps | BS464, 20 Medi-Preps |
|-----------------------|-----------------------------|-----------------------------|
| Solution I | 98 ml | 196 ml |
| Solution II | 200 ml | 2x200 ml |
| Solution III | 2x175 ml | 4x175 ml |
| Wash Solution | 24 ml | 48 ml |
| Elution Buffer | 20 ml | 40 ml |
| RNase A (10 mg/ml) | 2 ml | 2x2 ml |
| EZ-200 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% of ethanol to 24 ml Wash Solution for BS463, add 192 ml of 100% ethanol to 48ml Wash Solution for BS464. 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. Solution I should be stored at 4°C for frequent use ,or stored at -20 °C for longer period use.

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 200ug of plasmid DNA. Bacterial cultures are lysed and the lysates are cleared by routine methods (Solution I, II, III). Plasmid DNA from the cleared lysates is selectively adsorbed in EZ-200 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 30-40 minutes.
- Preparation of high quality plasmid DNA from culture. Purified DNA can be used in any downstream applications such as sequencing, transformation, restriction enzymatic and digestion.
- High yields (80-90%) and Reproducible.
- No phenol / chloroform extraction; No CsCl centrifugation; No ethanol precipitation



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Procedure for Purification of Plasmid DNA

1. Add 200ml overnight culture to a appropriate centrifuge tube and centrifuge at 6,000 rpm for 10 minutes. Drain the liquid completely.
2. Add 8 ml of Solution I to the pellet. Mix gently and keep for 2 minute.
3. Add 16 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 28 ml of Solution III, and mix gently. Incubate at RT for 2 minutes.
5. Spin at 10,000 rpm for 10 minutes.
6. Place EZ-200 spin 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 pre-warmed 50 ml centrifuge tube. Add 1ml 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.
11. Add additional 1 ml of Elution Buffer into center of EZ-200 spin column and spin at 8000 rpm for 2 minutes. Purified plasmid DNA can immediately be used or be stored at -20°C freezer.

Note: It is important to add the Elution Buffer into the center part of EZ-200 spin column. Elution Buffer at 55-80 °C could increase elution efficiency. Two times elution is recommended.