PRODUCT INFORMATION

EZ-10 96-WELL SPIN COLUMN PLATE PLASMID DNA MINIPREPS

Components	BS415 , 5 Plates	BS416 , 10 Plates
RNase A(10mg/ml)	1.2ml	2x1.2ml
Solution I(A)	60ml	120ml
Solution II(B)	120ml	240ml
Solution III	210ml	2x210ml
Wash Solution(C)	5x24ml	5x48ml
Elution Buffer(D)	30ml	60ml
96 Filter Plate	5	10
EZ-10 96 Well Plate	5	10
Deep Well Collection Plate	15	30
96 Storage Plate	5	10
Sealing Film	25	50
Protocol	1	1

(A) Before use, add RNase A to Solution I. Solution I should be stored at 4°C for frequent use, or stored at -20°C if not use for a long period.
(B) Solution II may form a precipitate upon storage. If necessary, dissolve the precipitate by warming at 37°C.
(C) Before use, add 192 ml of 96-100% of ethanol to 48 ml Wash Solution. If volume of Wash Solution is not sufficient due to leaking during transportation, it is necessary to re-measure its volume, and determine how many milliliters of ethanol to be added accordingly (volume of added ethanol: volume of Wash Solution=4:1).
(D) Elution Buffer is 2.5 mM Tris-HCl pH8.0~8.5. Although TE buffer pH 8.0 or water can be used, yield is generally 20% low.

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

Note: The purification method is based on centrifugation. There is a minimum height requirement of 750mm for apparatus to hold the assembly -- filter plate and deep well plate.

Principle

The EZ-10 96-well spin column plate plasmid DNA purification kit provides a simple, efficient and automated high throughput method for Mini plasmid DNA purifications. Plasmid DNA is selectively adsorbed in silica gel-based EZ-10 columns in the 96-well plate and other impurities such as proteins, salts and nucleotides are washed away. Plasmid DNA can be eluted in a small volume of Tris buffer.
Note: This kit is used for preparation of up to 10 ug of pure plasmid DNA in each well.

Applications

- Plasimid DNA purification

PRODUCT INFORMATION

Features

- Fast. Entire procedure takes 60 minutes.
- Preparation of high quality plasmid DNA from bacterial culture. Purified DNA can be used in any downstream applications such as sequencing, transformation, restriction enzymatic digestion, and ligation.
- High yields (>80%) and reproducible.
- No phenol / chloroform extraction or ethanol precipitation.

Quality Control
All components in the kit are tested in purification of 96 x 10 µg pUC 18 DNA from overnight culture.

Procedure for Purification of Plasmid DNA

1. Fill each well of a **Deep Well Collection** Plate with 1.3 ml of growth medium containing the appropriate selective agent. Inoculate each well from a single bacterial colony. Incubate the cultures for overnight or 20-24 hours at 37°C with shaking at 300 rpm.
2. Harvest the bacterial cells in the Deep Well Collection Plate by centrifugation for 5 min at 1500rpm in a centrifuge with a rotor for microtiter plates. The Deep Well Collection Plate should be covered with adhesive tape during centrifugation. Remove media by inverting the Deep Well Collection Plate.

Note: To remove the media, peel off the tape and quickly invert the Deep Well Collection Plate over a waste container. Tap the inverted Deep Well Collection Plate firmly on a towel to remove any remaining droplets of medium.

3. Resuspend each well of the bacterial cells in the Deep Well Collection Plate in 100 ul Solution I. Tape the Deep Well Collection Plate with sealing film. Mix by vortexing , and keep for 3 minutes.

Note: Ensure that RNase A has been added to Solution I. The pelleted cells in the Deep Well Collection Plate be resuspended completely leaving no cell clumps.

4. Add 200 ul of Solution II to each well, seal the Deep Well Collection Plate with new tape, mix gently but thoroughly by inverting 10 times and keep for 2 minutes.

Note: Do not vortex at this step, as this may cause shearing of the bacterial genomic DNA. Do not incubate for more than 5 minutes. Additional incubation can result in increased levels of open circular plasmid DNA. At the end of the incubation, the lysate should appear viscous and free of bacterial cell clumps. Avoid extended exposure of Solution II to air since CO_2 can reduce the effectiveness of this solution.

5. Add 350ul Solution III to each well, seal the Deep Well Collection Plate with new tape, and mix immediately by inverting 10 times and keep for 3 minutes.

Note: Gently inverting the taped Deep Well Collection Plate 10 times to ensure uniform precipitation.

6. Spin down at 8,000 rpm for 15 minutes.

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7. Remove the tape. Assemble 96-filter plate on top of a new Deep Well Collection Plate. Pipet the lysate from step 6 (650ul) into the center wells of 96-filter plate. Seal the plate with a new tape.
8. Centrifuge at 3,000rpm for 5min.
9. Place an EZ-10 96-well spin column plate on the top of a new Deep Well Collection.
10. Transfer the above supernatants (clear lysate) from step 8 into EZ-10 96 well plate using an 8-channl pipette. Centrifuge at 3,000 rpm for 5 minutes.
11. Discard the flow-through in the Deep Well Collection. Add 500 ul of Wash Solution to each well of EZ-10 96-well plate, and centrifuge at 6,000 rpm for 5 minutes.
12. Repeat wash procedure in step 9.
13. Discard the flow-through in the deep well collection plate. Spin at 6,000 rpm for additional 5 minutes to remove residual Wash Solution.
14. Place the EZ-10 96-well spin column on the top of a 96-well storage plate. Add 50 ul of Elution Buffer into the center part of the membrane of each well and incubate at 37- 50°C for 2 minutes. Spin at 6,000 rpm for 5 minutes.
15. Plasmid DNA is ready for use or store at –20°C freezer.

Note: It is important to add the Elution Buffer into the center part of each well.

Troubleshooting:

1. RNA contamination
   a) RNase A digestion might be insufficient. Check culture volume against recommended volumes, and reduce if necessary. If RNase A solution is kept for more than 6 months, add more RNase A.
   b) Prolong the standing time after adding supernatant to the EZ-10 96-well plate.
   c) It is extremely important to add the Elution Buffer to the center of the EZ-10 96-well plate. Pre-warming the Elution Buffer to 80°C can improve recovery yield of DNA. Or after adding the Elution Buffer to the EZ-10 96-well plate, incubate at 55°C to 60°C for 3–5 minutes.
2. Low yield of plasmid DNA
   Alkaline lysis was inefficient: If cells have grown to very high densities or a larger amount of cultured medium than recommended was used, the ratio of biomass to lysis reagent is shifted. This may result in poor lysis conditions, because the volumes of solution I, II and III are efficiently. Reduce culture volume or increase volumes of Solution I, II and III.