PRODUCT INFORMATION

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EZ96-204 96-well Plasmid DNA Mini Prep kit

Components	EZ96-204, 2 Plates	EZ96-204, 5 Plates
RNase A(10mg/ml)	0.5 ml	1.2 ml
Solution I[a]	25 ml	60 ml
Solution II[b]	50 ml	120 ml
Solution III	90 ml	210 ml
Wash Solution[c]	2x35 ml	4x48 ml
Elution Buffer[d]	15 ml	30 ml
EZ96FTP (96 well Filter Plate)	2	5
EZ96DBP plates (DNA Binding plates)	2	5
EZ96DWP plate (2ml deep well collection plates)	4	10
96 Storage plate	2	5
Sealing film	10	25
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 being used for a long period of time.
[b]Solution II may form a precipitate upon storage. If necessary, dissolve the precipitate by warming at 37°C.
[c]Before use, add 140 ml of 96-100% ethanol to each 35-ml Wash Solution.
[d]Elution Buffer is 2.5 mM Tris-HCl, pH 8.0~8.5. TE buffer (pH 8.0) or water may be used instead.

Principle

The 96-well plasmid DNA miniprep kit provides a simple, efficient and automated high throughput method for Mini plasmid DNA purification. Plasmid DNA is selectively adsorbed by silica membrane in the well of the DNA Binding Plate (EZ96DBP). Impurities such as protein, 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 μg of pure plasmid DNA in each well.

Applications

Plasmid DNA purification

Features

- Fast. Entire procedure takes 60 minutes.
- Preparation of high quality plasmid DNA from bacterial culture. Purified DNA can be used in any 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 (Vacuum Manifold can also be used)

1. Fill each well of a Deep Well 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.
   NOTE: The wells in the block may be protected against spill-over by converting the block with a plastic lid or adhesive tape. If non-porous tape is used, pierce 2-3 holes in the tape with a needle for aeration.
2. Harvest the bacterial cells in the Deep Well Plate by centrifugation for 5 min at 5,788xg in a centrifuge with a rotor for microtiter plates. The Deep Well Plate should be covered with adhesive tape during centrifugation. Remove media by inverting the Deep Well Plate.
   Note: To remove the media, peel off the tape and quickly invert the Deep Well Plate over a waste container. Tap the inverted Deep Well Plate firmly on a towel to remove any remaining droplets of medium.
3. Resuspend each well of the bacterial cells in the Deep Well Plate in **100 μl** Solution I. Tape the Deep Well 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 Plate should be resuspended completely leaving no cell clumps.

4. Add **200 µl** of Solution II to each well, seal the Deep Well 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 CO2 can reduce the effectiveness of this solution.
5. Add **350 µl** Solution III to each well, seal the Deep Well Plate with new tape, and mix immediately by inverting 10 times and keep for 3 minutes.
   Note: Gently inverting the taped Deep Well Plate 10 times to ensure uniform precipitation.
6. Spin down at 4,500xg for 15 minutes.
7. Remove the tape. Assemble 96 well Filter Plate (EZ96FTP) on top of a new Deep Well Plate. Pipette the lysate from step 6 (650 µl) into the center of wells of 96 filter plate. Seal the plate with a new tape.
   NOTE: This step is to efficiently remove any cell debris from lysate. Desired DNA will be collected in the Deep Well Plate.
8. Centrifuge at 5,700xg for 5min.
9. Place a DNA Binding Plate (EZ96DBP) on top of a new Deep Well Plate.
10. Transfer the above supernatants (clear lysate) from step 8 into Binding Plate using multiple channel pipette. Centrifuge at 5,700xg for 5 minutes.
11. Discard the flow-through in the Deep Well Plate. Add **500 µl** of Wash Solution to each well of DNA Binding Plate and centrifuge at 5,700xg for 5 minutes. Repeat wash procedure.
12. Discard the flow-through in the deep well collection plate. Spin at 5,700xg for additional 5 minutes to remove residual Wash Solution.
13. Place the DNA Binding Plate on top of a 96-well storage plate. Add 50 µl of Elution Buffer into the center part of the membrane of each well and incubate at 37- 50°C for 2 minutes. Spin at 5,700xg for 5 minutes.
    NOTE: 96-well Storage Plate is very fragile and needs to be place on top of a Deep Well Plate for support during centrifugation.
14. 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 adjust if necessary. If RNaseA solution is kept for more than 6 months, add more RNase A.
   b) Prolong the standing time after adding supernatant to the DNA Binding Plate.
   c) It is extremely important to add the Elution Buffer to the center of the DNA Binding Plate. Prewarming the Elution Buffer to 80°C can improve recovery yield of DNA. Or after adding the Elution Buffer to the DNA Binding 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 insufficient. Reduce culture volume or increase volumes of Solution I, II and III.

3. For optimal results in downstream DNA sequencing, an additional washing step is recommended.