Plasmid Mini Kit

Cat.No.: HYC211

Product Description

This kit is designed for plasmid DNA extraction from 1–5 mL bacterial cultures. Based on alkaline lysis for cell lysis, it incorporates proprietary silica-membrane binding technology and optimized reagent formulations. Under high-salt conditions, the spin column enables efficient, specific capture of plasmid DNA, with a maximum binding capacity of 30 μg plasmid DNA per column, while maximally eliminating proteins, genomic DNA, RNA and other contaminants. Purified plasmid DNA is directly compatible for downstream applications including cell transfection, PCR, restriction digestion, DNA sequencing and ligation.

Components

Components	200 T
Buffer P1	60 mL
Buffer P2	60 mL
Buffer N3	80 mL
Buffer PB	35 mL
Buffer PW*	25 mL
Buffer EB	30 mL
RNase A (10 mg/mL)	600 μL
Spin Columns DM with Collection Tubes	200

Storage

All components can be stably stored in a dry place at room temperature (15-30℃).

Required Reagents and Equipment

- Absolute Ethanol

Instructions

Preparations

- Before first use, transfer the entire RNase A solution into Buffer P1 and mix well. Store the mixed buffer at 2–8 °C; equilibrate to room temperature prior to use.
- Add absolute ethanol to Buffer PW as indicated on the bottle label before initial use.
- If precipitates form in Buffer P2, Buffer N3 or Buffer PB, incubate the buffer in a 37 °C water bath for several minutes to clarify. Do not shake Buffer P2 vigorously.
- Avoid direct contact with Buffer P2, Buffer N3 and Buffer PB. Cap all bottles tightly immediately after use.
- The yield and purity of isolated plasmid DNA depend on bacterial culture density, strain species, plasmid size, plasmid copy number and other factors.

Procedures

1. Transfer 1–5 mL overnight bacterial culture into a self-supplied centrifuge tube. Centrifuge at 13,000 rpm (~16,200 × g) for 30 sec to pellet cells, then thoroughly discard all supernatant.
2. Add 250 µL Buffer P1 (confirm RNase A has been added beforehand) to the cell pellet. Resuspend the pellet completely by pipetting or vortexing.
   Note: Incomplete resuspension of cell clumps compromises lysis efficiency and reduces plasmid yield and purity.
3. Add 250 µL Buffer P2 to the tube. Mix gently by inverting the tube 4–6 times for full lysis; the solution will turn clear and viscous.
   Note: Mix gently without vigorous vortexing to prevent genomic DNA fragmentation and gDNA contamination in purified plasmids. Complete this step within 5 min to avoid plasmid degradation.
4. Add 350 µL Buffer N3 and immediately mix gently by inverting 8–10 times until white flocculent precipitates form. Centrifuge at 13,000 rpm for 5 min.
   Note: Mix right after adding Buffer N3 to prevent localized precipitation.
5. Transfer the supernatant from Step 4 into a Spin Column DM fitted with a collection tube. Centrifuge at 13,000 rpm for 30 sec. Discard flow-through and reinsert the spin column back into the collection tube.

6. Add 150 μL Buffer PB to the spin column, then centrifuge at 13,000 rpm for 30 sec.
7. Add 400 μL Buffer PW (confirm absolute ethanol has been added beforehand). Centrifuge at 13,000 rpm for 1 min and discard flow-through.
8. Centrifuge the empty spin column at 13,000 rpm for 2 min to remove residual liquid and evaporate trace ethanol completely.

   Note: Perform this spin after discarding flow-through. Residual ethanol impairs plasmid quality and causes sample well leakage during agarose gel electrophoresis.
9. Place the spin column into a new self-supplied centrifuge tube. Add 50–100 μL Buffer EB to the center of the silica membrane, incubate at room temperature for 2 min, then centrifuge at 13,000 rpm for 1 min to elute plasmid DNA. Store purified plasmids at −20 °C.

   Notes:

   1) For higher recovery, reload the eluate back onto the spin column, incubate 2 min at room temperature, and re-centrifuge at 13,000 rpm for 1 min to collect more plasmid DNA.

   2) Preheat Buffer EB to 65–70 °C in a water bath to improve elution yield for low-copy plasmids or plasmids larger than 10 kb.