EndoFree Plasmid Mini Kit

Cat.No.: HYC214

Product Description

Endotoxin is a prevalent contaminant during plasmid purification. Eukaryotic cells are highly sensitive to endotoxin; endotoxin-contaminated plasmids will drastically reduce transfection efficiency in eukaryotic cells. This kit delivers a simple, rapid and high-performance method for endotoxin-free plasmid isolation. It removes endotoxin to the greatest extent while effectively eliminating genomic DNA, RNA, protein and other impurities, with straightforward operation. This kit is suitable for plasmid extraction from 1–5 mL bacterial cultures. Built upon alkaline lysis, it utilizes innovative silica membrane technology to capture plasmid DNA efficiently and specifically, with a maximum binding capacity of 40 μg plasmid DNA per spin column. A specialized buffer system and dedicated endotoxin removal filter column are integrated to thoroughly clear endotoxin, protein and other contaminants. Purified plasmids feature high purity and consistent quality, making them ideal for cell transfection. The product is also compatible with downstream applications including DNA sequencing, PCR, PCR-based mutagenesis, in vitro transcription, bacterial transformation and restriction endonuclease digestion.

Components

Components	50 T
Buffer P1	15 mL
Buffer P2	15 mL
Buffer E3	15 mL
Buffer PS	15 mL
Buffer PW*	10 mL
EndoFree Buffer EB	10 mL
RNase A (10 mg/mL)	150 μL
Endo-Remover FM with Collection Tubes	50
Spin Columns DM with Collection Tubes	50

Storage

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

Required Reagents and Equipment

- Absolute Ethanol
- Isopropanol

Instructions

Preparations

- Buffer P1 supplemented with RNase A remains stable for 6 months when stored at 2–8 °C.
- Prior to first use, transfer the entire RNase A solution into Buffer P1 and mix thoroughly. Store the mixture at 2–8 °C, and equilibrate to room temperature before use.
- Add absolute ethanol to Buffer PW as instructed on the bottle label before initial use.
- Check Buffer P2 and Buffer E3 for crystals or precipitates prior to use. If solids are observed, incubate the buffers in a 37 °C water bath for several minutes until clear.
- Avoid direct contact with Buffer P2 and Buffer E3; tightly cap the bottles immediately after use.
- The yield and purity of purified plasmid DNA depend on bacterial culture density, strain type, plasmid size, plasmid copy number and other relevant factors.

Procedures

1. Transfer 1–5 mL overnight bacterial culture to a self-supplied centrifuge tube. Centrifuge at 13,000 rpm (~16,200 × g) for 30 seconds to pellet bacteria, then remove all supernatant thoroughly.
2. Add 250 µL Buffer P1 (confirm RNase A has been added in advance) to the cell pellet. Fully resuspend the pellet by pipetting or vortexing.
   Note: Incomplete dispersion of cell clumps impairs lysis and reduces plasmid yield and purity.
3. Add 250 µL Buffer P2 and mix gently by inverting the tube 8–10 times for complete lysis. Incubate at room temperature for 3–5 min; the solution will turn clear and viscous.
   Note: Mix gently without vigorous vortexing to prevent genomic DNA shearing and gDNA contamination. If the solution fails to clear, the bacterial load is excessive and the starting culture volume should be reduced.

4. Add 250 µL Buffer E3 and immediately invert the tube 8–10 times to mix, forming white flocculent precipitates. Incubate at room temperature for 5 min, then centrifuge at 13,000 rpm for 5 min. Transfer the supernatant to an Endo-Remover FM filter column, centrifuge at 13,000 rpm for 1 min, and collect the flow-through in a new self-supplied centrifuge tube.
   Note: Mix immediately after adding Buffer E3 to avoid localized precipitation.
5. Add isopropanol equal to 0.3 times the volume of the supernatant, and mix by tube inversion.
6. Column equilibration: Add 200 µL Buffer PS to a Spin Column DM seated in a collection tube. Centrifuge at 13,000 rpm for 1 min, discard flow-through, and reinsert the spin column into the collection tube.
7. Load the mixture of filtrate and isopropanol from Step 5 onto the equilibrated spin column (with collection tube attached).
8. Centrifuge at 13,000 rpm for 1 min, discard flow-through, and replace the spin column into the collection tube.
   Note: The maximum loading volume of the spin column is 750 µL. Split large-volume samples into multiple loads if needed.
9. Add 750 µL Buffer PW (verify absolute ethanol has been supplemented before use). Centrifuge at 13,000 rpm for 1 min and discard flow-through.
10. Reinsert the spin column into the collection tube and centrifuge at 13,000 rpm for 1 min.
    Note: This spin removes residual ethanol, which inhibits downstream enzymatic reactions such as restriction digestion and PCR.
11. Transfer the spin column to a fresh collection tube. Add 50–100 µL Endo-Free Buffer EB to the center of the silica membrane, incubate at room temperature for 2–5 min, then centrifuge at 13,000 rpm for 2 min to elute plasmid DNA. Store purified plasmids at −20 °C.
    Notes:
    1) **To improve recovery, reload the eluate back onto the spin column, incubate for another 2–5 min at room temperature, and re-centrifuge at 13,000 rpm for 2 min to collect more plasmid DNA.**
    2) **Preheat Endo-Free Buffer EB in a 65–70 °C water bath to boost extraction efficiency for low-copy plasmids or plasmids larger than 10 kb.**