EndoFree Plasmid Maxi Kit

Cat.No.: HYC213

Product Description

Endotoxin is a common contaminant in plasmid purification. Eukaryotic cells are highly sensitive to endotoxin, and endotoxin-contaminated plasmids will significantly reduce eukaryotic cell transfection efficiency. Based on alkaline lysis for cell lysis, this kit adopts proprietary silica membrane adsorption technology to bind plasmid DNA efficiently and specifically. Specially formulated endotoxin-removal Buffer ER1 and Endo-Remover FQ are used to effectively remove endotoxin, protein and other contaminants. A dedicated HYBlue indicator is also provided to guarantee efficient plasmid purification.

This kit handles 100–300 mL bacterial culture per preparation, delivering plasmids with high purity and high yield, yielding up to 2 mg transfection-grade plasmid DNA. The purified plasmids are also applicable to DNA sequencing, PCR, in vitro transcription, restriction endonuclease digestion and other experiments.

Components

Components	10 T
Buffer P1	125 mL
Buffer P2	125 mL
Buffer E3	125 mL
Buffer PS	30 mL
Buffer PW*	50 mL
EndoFree Buffer EB	30 mL
RNase A (10 mg/mL)	2 mL
Buffer ER1	50 mL
Buffer PB	100 mL
HYBlue	3 mL
Spin Columns DZ with Collection Tubes	2×5 sets
Endo-Remover FQ	2×5 sets
Centrifuge Tubes (50 mL)	2×5 sets

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

1. Buffer P1 supplemented with RNase A is stable for 6 months when stored at 2–8 °C.
2. Prior to first use, add absolute ethanol to Buffer PW as instructed on the bottle label. Transfer the entire RNase A solution into Buffer P1 and mix thoroughly, then store the mixture at 2–8 °C. Equilibrate to room temperature before use.
3. Inspect Buffer P2, Buffer E3 and Buffer ER1 for crystals or precipitates before use. If solids are observed, incubate the buffers in a 37 °C water bath for several minutes until clear.
4. Caution: Buffer P2 and Buffer E3 contain irritants. Wear gloves during handling and tightly cap all bottles immediately after use.
5. For optimal results, load the mixture onto Buffer PS-equilibrated spin columns after a 15–30 min standing period. Columns are not recommended for use after standing longer than 30 minutes.
6. Instructions for HYBlue Indicator: Mix HYBlue and Buffer P1 at a volume ratio of 1:100 and homogenize thoroughly. After adding Buffer P2 and thorough mixing, a uniform clear blue solution indicates complete bacterial lysis. Subsequent addition and full mixing of Buffer E3 yields a colorless transparent solution with suspended white flocculent precipitates, confirming sufficient neutralization and renaturation.

Procedures (Fast Version)

1. Transfer 150 mL overnight bacterial culture into self-supplied centrifuge tubes. Centrifuge at 12,000 × g for 2–3 min to pellet bacteria, then thoroughly remove all supernatant.
   Note: Refer to the appendix for applicable bacterial culture volumes.
2. Add 12 mL Buffer P1 (confirm RNase A has been pre-added) and 120 µL HYBlue to the cell pellet. Fully resuspend the pellet by pipetting or vortexing.

Note: Incomplete dispersion of cell clumps will compromise lysis efficiency and reduce plasmid yield and purity.

3. Add 12 mL Buffer P2, mix gently by inverting the tube 8–10 times for full bacterial lysis, and incubate at room temperature for 5 min. The solution will turn clear and viscous.

   Notes:

   1) Mix gently without vigorous vortexing to prevent genomic DNA shearing and gDNA contamination in purified plasmids.

   2) If the solution fails to turn clear, the bacterial load is excessive with incomplete lysis; reduce the starting culture volume accordingly.

   3) A uniform blue solution without obvious white floccules after mixing with Buffer P2 indicates complete bacterial lysis.

4. Add 12 mL Buffer E3 and immediately invert the tube 8–10 times until white precipitates form, then incubate at room temperature for 5 min.

   Note: Mix instantly after adding Buffer E3 to avoid localized precipitation. A colorless transparent solution suspended with loose curd-like white precipitates signifies sufficient neutralization.

5. Column Equilibration: Add 2 mL Buffer PS to Spin Columns DZ placed in collection tubes. Centrifuge at 12,000 × g for 2 min, discard flow-through, and retain the equilibrated spin columns for Step 8.

   Note: Let equilibrated columns stand for 15–30 min (do not exceed 30 min) before Step 8 to optimize column performance and plasmid yield.

6. After finishing Step 4, centrifuge the mixture at 12,000 × g for 10 min. Transfer all supernatant to an Endo-Remover FQ filter; avoid loading large precipitate clumps. Slowly push the plunger for filtration and collect filtrate in a clean self-supplied 50 mL centrifuge tube.

7. Add isopropanol equal to 0.3 times the volume of the filtrate, and mix by tube inversion.

8. Load the filtrate-isopropanol mixture onto the equilibrated Spin Column DZ (fitted with a collection tube). Centrifuge at 12,000 × g for 2 min, discard flow-through, and reinsert the spin column into the collection tube.

   Note: The maximum loading volume of each spin column is 15 mL; load in multiple batches if the sample volume exceeds the limit. For centrifuges with steep-angle rotors, load no more than 10 mL per column to prevent liquid leakage.

9. Add 10 mL Buffer PW (verify absolute ethanol has been supplemented beforehand) to the spin column. Centrifuge at 12,000 × g for 2 min, discard flow-through, and reinsert the spin column.
10. Repeat Step 9.
11. Place the spin column back into the collection tube and centrifuge at 12,000 × g for 5 min. Discard flow-through and leave the spin column at room temperature for 5 min to fully evaporate residual wash buffer.

    Note: This step removes residual ethanol, which inhibits downstream enzymatic reactions including restriction digestion and PCR.
12. Transfer the spin column to a fresh centrifuge tube. Add 1–3 mL EndoFree Buffer EB to the center of the silica membrane, incubate at room temperature for 2–5 min, then centrifuge at 12,000 × g for 5 min to collect eluted plasmid DNA. Store purified plasmids at −20 °C.

    Notes:

    1) To improve recovery, reload the eluate onto the spin column, incubate at room temperature for another 2–5 min, and re-centrifuge at 12,000 × g for 5 min to harvest more plasmid DNA.

    2) Preheat EndoFree Buffer EB in a 65–70 °C water bath to boost extraction efficiency for low-copy plasmids or plasmids larger than 10 kb.

Procedures (Enhanced Endotoxin Removal Version)

1. Transfer 150 mL overnight bacterial culture into self-supplied centrifuge tubes. Centrifuge at 12,000 × g for 2–3 min to pellet bacteria, then thoroughly remove all supernatant.

    Note: Refer to the appendix for applicable bacterial culture volumes.
2. Add 12 mL Buffer P1 (confirm RNase A has been pre-added) and 120 µL HYBlue to the cell pellet. Fully resuspend the pellet by pipetting or vortexing.

    Note: Incomplete dispersion of cell clumps will compromise lysis efficiency and reduce plasmid yield and purity.
3. Add 12 mL Buffer P2, mix gently by inverting the tube 8–10 times for full bacterial lysis, and incubate at room temperature for 5 min. The solution will turn clear and viscous.

    Notes:

    1) Mix gently without vigorous vortexing to prevent genomic DNA shearing and gDNA contamination in purified plasmids.

2) **If the solution fails to turn clear, the bacterial load is excessive with incomplete lysis; reduce the starting culture volume accordingly.**
3) **A uniform blue solution without obvious white floccules after mixing with Buffer P2 indicates complete bacterial lysis.**

4. Add 12 mL Buffer E3 and immediately invert the tube 8–10 times until white precipitates form, then incubate at room temperature for 5 min.
5. Column Equilibration: Add 2 mL Buffer PS to Spin Columns DZ placed in collection tubes. Centrifuge at 12,000 × g for 2 min, discard flow-through, and retain the equilibrated spin columns for Step 9.

   Note: Let equilibrated columns stand for 15–30 min (do not exceed 30 min) before Step 9 to optimize column performance and plasmid yield.
6. After finishing Step 4, centrifuge the mixture at 12,000 × g for 10 min. Transfer all supernatant to an Endo-Remover FQ filter; avoid loading large precipitate clumps. Slowly push the plunger for filtration and collect filtrate in a clean self-supplied 50 mL centrifuge tube.

   Note: Mix instantly after adding Buffer E3 to avoid localized precipitation. A colorless transparent solution suspended with loose curd-like white precipitates signifies sufficient neutralization.
7. Add Buffer ER1 equal to 0.1 times the filtrate volume, then mix by tube inversion.
8. Add isopropanol equal to 0.3 times the filtrate volume, then mix by tube inversion.
9. Load the mixture from Step 8 onto the equilibrated Spin Column DZ (fitted with a collection tube). Centrifuge at 12,000 × g for 2 min, discard flow-through, and reinsert the spin column into the collection tube.

   Note: The maximum loading volume of each spin column is 15 mL; load in multiple batches if the sample volume exceeds the limit. For centrifuges with steep-angle rotors, load no more than 10 mL per column to prevent liquid leakage.
10. Optional Step: Add 10 mL Buffer PB to the spin column. Centrifuge at 12,000 × g for 2 min, discard flow-through, and reinsert the spin column.

    Note: Use Buffer PB when the OD_{600} reading exceeds 400 to avoid reduced plasmid purity.

11. Add 10 mL Buffer PW (verify absolute ethanol has been supplemented beforehand) to the spin column. Centrifuge at 12,000 × g for 2 min, discard flow-through, and reinsert the spin column.
12. Repeat Step 11.
13. Place the spin column back into the collection tube and centrifuge at 12,000 × g for 5 min. Discard flow-through and leave the spin column at room temperature for 5 min to fully evaporate residual wash buffer.

    Note: This step removes residual ethanol, which inhibits downstream enzymatic reactions including restriction digestion and PCR.
14. Transfer the spin column to a fresh centrifuge tube. Add 1–3 mL EndoFree Buffer EB to the center of the silica membrane, incubate at room temperature for 2–5 min, then centrifuge at 12,000 × g for 5 min to collect eluted plasmid DNA. Store purified plasmids at −20 °C.

    Notes:

    1) To improve recovery, reload the eluate onto the spin column, incubate at room temperature for another 2–5 min, and re-centrifuge at 12,000 × g for 5 min to harvest more plasmid DNA.

    2) Preheat EndoFree Buffer EB in a 65–70 °C water bath to boost extraction efficiency for low-copy plasmids or plasmids larger than 10 kb.