EasyPure® HiPure Plasmid MaxiPrep Kit

Cat. No. EM121

Storage: RNase A at -20°C for two years; others at room temperature (15-25°C) for one year

Description

EasyPure® HiPure Plasmid MaxiPrep Kit uses a modified alkaline lysis method to isolate high quality plasmid DNA from ≤ 500 ml (LB) of bacterial culture. Unique formulated lysis buffer and neutralization buffer permit complete bacterial cell lysis and neutralization. Endotoxin is removed by a simple incubation on column with a novel buffer. The purified DNA is suitable for a variety of molecular biology applications including restriction
enzyme digestion, ligation, transformation, DNA sequencing, and transfection.

- Fast: the whole procedure can be performed in one hour.
- Simple: endotoxin is removed on column.
- High yield: DNA yield up to 1 mg.

Kit Contents

Component	EM121-01 (10 rxns)
Resuspension Buffer (RB)	120 ml
Lysis Buffer (LB)	120 ml
Neutralization Buffer (NB)	160 ml
ToxinOut Buffer (TB)	60 ml
Wash Buffer (WB)	25 ml
Elution Buffer (EB)	25 ml
RNase A (10 mg/ml)	1.2 ml
Maxi-Plasmid Spin Columns with Collection Tubes	10 each

Procedures

Prior to use, add RNaseA to RB, store at 2-8°C; add 100 ml of 96-100% ethanol to WB.

Bacterial cell culture (LB)	RB	LB	NB
≤ 100 ml	5 ml	5 ml	7 ml
100 ml-200 ml	10 ml	10 ml	14 ml
200 ml-300 ml	15 ml	15 ml	21 ml
300 ml-400 ml	20 ml	20 ml	28 ml
400 ml-500 ml	25 ml	25 ml	35 ml

1. Centrifuge overnight bacterial cell culture (refer to above table) at 12,000×g for 2 minutes and discard the supernatant.
2. Add 5/10 ml of colorless RB (containing RNase A). Mix thoroughly by vortexing.
3. Add 5/10 ml of blue LB, mix by gently inverting the tube 4-6 times to completely lyse cells. Incubate at room temperature for 5 minutes (lysate should change color to blue).
4. Add 7/14 ml of yellow NB, mix by gently inverting the tube 5-6 times. Incubate at room temperature for 2 minutes (lysate should change color to yellow).
5. Centrifuge at 12,000×g for 15 minutes, gently transfer the supernatant to a spin column (if more than 50 ml, repeat the transfer after step 6).
6. Centrifuge at 8,000×g for 2 minutes and discard the flow-through.
7. Add 5 ml of TB, incubate at room temperature for 10 minutes. Centrifuge at 8,000×g for 2 minutes and discard the flow-through.

8. Add 3/5 ml of WB, centrifuge at 8,000×g for 2 minutes and discard the flow-through.
9. Repeat step 8 once.
10. Centrifuge at 8,000×g for 5 minutes to completely remove the remaining WB.
11. Incubate at room temperature for 10 minutes to evaporate ethanol.
12. Place the spin column in a clean 50 ml centrifuge tube, add 1-2 ml of of EB or sterile, distilled water (pH >7.0) directly to the center of the column matrix (for higher yield, use prewarmed (60-70°C) EB or distilled water).
13. Centrifuge the column at 8,000×g for 2 minutes to elute DNA.
14. Isolated plasmid DNA can be stored at -20°C.

Optional (to futher concentrate DNA)

1. Transfer eluent to a microcentrifuge tube, add 1/10 volume of NaAC (3 M, pH 5.2) and 7/10 volume of isopropanol (at room temperature). Mix thoroughly and incubate at room temperature for 5 minutes.
2. Centrifuge at 12,000×g for 10 minutes and discard the supernatant.
3. Add 1 ml of 70% ethanol (room temperature), centrifuge at 12,000×g for 10 minutes and discard the supernatant.
4. Air-dry the pellet for 5-10 minutes. Add appropriate volume of EB to dissolve the pellet.

Notes

- All centrifugation steps are carried out at room temperature.
- After adding LB or NB, don't mix by vortexing. Vigorous mix may result in genome contamination.
- Add the whole volume of RNase A (supplied with this kit) into RB solution, mix thoroughly and store at 2-8°C.
- Prior to use, check whether the LB is cloudy or not, if it is cloudy, heat it in 37°C water bath to completely dissolve it. Close the cap immediately after each use to avoid pH change.
- Make sure to use the right cell culture volume. Too much cell culture can result in incomplete lysis, which will affect plasmid DNA yield and purity.
- 200 ml LB Media is considered as 1 rxn.

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