AllPure Plasmid MiniPrep Kit

Cat. No. ABTGNA005

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

Description

AllPure Plasmid MiniPrep Kit provides an efficient way to isolate high quality plasmid DNA from ≤20 ml (LB) or ≤4 ml (AllBio customized Plasmid Culture Media) bacterial cell culture, with DNA yield up to 40 μg. Unique formulated lysis buffer and neutralization buffer permit error-free visual identification of complete bacterial cell lysis and neutralization. The purified plasmid DNA is suitable for a variety of molecular biology applications, including restriction enzyme digestion, ligation, transformation, DNA sequencing and transfection.

Kit Contents

Component	50 rxns	200 rxns
Resuspension Buffer (RB)	15 ml	60 ml
Lysis Buffer (LB, Blue)	15 ml	60 ml
Neutralization Buffer (NB, yellow)	20 ml	80 ml
Wash Buffer (WB)	10 ml	2x20 ml
Elution Buffer (EB)	5 ml	10 ml
RNase A (10 mg/ml) -20°C	150 μl	600 μl
Mini-Plasmid Spin Column with Collection Tubes	50 each	200 each

Procedures

1. Add overnight cultured bacterial suspension to a microcentrifuge tube.

LB Media	AllBio customized Plasmid Culture Media	RB	LB	NB
≤5 ml	≤1 ml	250 µl	250 µl	350 µl
5~10 ml	1~2 ml	500 µl	500 µl	700 µl
10~15 ml	2~3 ml	750 µl	750 µl	1050 µl
15~20 ml	3~4 ml	1000 µl	1000 µl	1400 µl

2. Centrifuge at 10,000×g for 1 minute. Discard the supernatant.
3. Add appropriate volume of RB (premixed with RNase A) to the cell pellet and resuspend it completely by pipetting.
4. Add appropriate volume of LB (Blue) , mix immediately and thoroughly by inverting the tube 4-6 times.
   (After this step, the lysate should change from opaque to bright blue. Proceed the following steps within 5 minutes after this step.)
5. Add appropriate volume of NB (Yellow), mix thoroughly by inverting the tube 4-6 times. The lysate will turn yellow when the neutralization is complete and a yellowish precipitate will form. Incubate the lysate at room temperature for 2 minutes.
6. Centrifuge at 12,000×g for 5 minutes. Transfer the supernatant into a spin column.
7. Centrifuge at 12,000×g for 1 minute. Discard the flow through.
8. Add 650 µl of WB (check to make sure that ethanol has been added.) to the column, Centrifuge at 12,000×g for 1 minute. Discard the flow through.
9. Centrifuge the empty column at 12,000×g for 1-2 minutes to remove residual WB completely.
10. Place the spin column in a clean microcentrifuge tube, add 30-100 µl of EB or sterile, distilled water (pH >7.0) directly to the center of the column matrix (for higher yield, preheat EB or water to 65°C). Incubate the column at room temperature for 1 minute. Centrifuge the column at 10,000×g for 1 minute to elute DNA. The isolated plasmid DNA is ready to use or can be stored at -20°C.

Notes

- All centrifugation steps are carried out at room temperature.
- Add the entire RNase A from its tube to one bottle of RB solution, mix well and store at 4°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. Tight the cap immediately after use to avoid pH change.
- Maximum DNA yield by this kit is 40 µg. If plasmid DNA yield is low, increase the volume of bacterial culture or use AllBio Plasmid Culture. Plasmid DNA yield from AllBio Plasmid Culture is much higher than that from LB medium.
- Use the amount of RB, LB and NB as suggested in the manual. Too much cell culture can result in incomplete lysis, which will affect plasmid DNA yield and the purity.