Plasmid MiniPrep Kit

Catalogue No.:abx098081

Plasmid MiniPrep Kit provides an efficient way to isolate high quality plasmid DNA from ≤ 20 ml (LB) or ≤ 4 ml bacterial cell culture, with DNA yield up to 40 µg. A uniquely formulated lysis buffer and neutralization buffer allows 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)	15 ml	60 ml
Neutralization Buffer (RB)	20 ml	80 ml
Wash Buffer (WB)	10 ml	2 × 20 ml
Elution Buffer (EB)	5 ml	10 ml
RNase A (10 mg/ml)	150 µl	600 µl
Mini-Plasmid Spin Columns with Collection Tubes	50	2 × 100

Target: Plasmid MiniPrep Kit

Storage: Store at room temperature (15-25 °C). Once prepared (see Assay Procedure), store the Working Resuspension Buffer between 2-8 °C.

Note: THIS PRODUCT IS FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC, THERAPEUTIC OR COSMETIC PROCEDURES. NOT FOR HUMAN OR ANIMAL CONSUMPTION.

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Directions for use:

Assay Procedure

1. Prepare the Working Resuspension Buffer by adding the entire RNase vial to the Resuspension Buffer bottle. **Mix well and store between 2-8 °C.**
2. Prepare the Working Wash Buffer by adding 100% ethanol to each Wash Buffer bottle: 40 ml (50 rxns); or 2 × 80 ml (200 rxns). Mix well.
3. Add overnight cultured bacterial suspension to a microcentrifuge tube. Measure the LB Medium volume and note down the reagent volumes required in the table below.

LB Medium	Working Resuspension Buffer	Lysis Buffer	Neutralization Buffer
≤ 5 ml	250 µl	250 µl	350 µl
5-10 ml	500 µl	500 µl	700 µl
10-15 ml	750 µl	750 µl	1050 µl
15-50 ml	1000 µl	1000 µl	1400 µl

4. Centrifuge at 10,000 × g for 1 minute. Discard the supernatant.
5. Add an appropriate volume of Working Resuspension Buffer (premixed with RNase A), according to the table above, to the cell pellet and resuspend it completely by pipetting.
6. Add an appropriate volume of Lysis Buffer (blue liquid), according to the table above. Mix immediately and thoroughly by inverting the tube 4-6 times. The lysate should change from opaque to bright blue.
7. Within 5 minutes after completing step 6, add an appropriate volume of Neutralization Buffer (yellow liquid), according to the table above. 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. Allow the lysate to stand at room temperature for 2 minutes.
8. Centrifuge at 12,000 × g for 5 minutes. Transfer the supernatant into a spin column.
9. Centrifuge at 12,000 × g for 1 minute. Discard the flow through.
10. Add 650 µl of Working Wash Buffer (with added ethanol) to the column, then centrifuge at 12,000 × g for 1 minute. Discard the flow through.
11. Centrifuge the empty column at 12,000 × g for 1-2 minutes to remove any residual Wash Buffer completely.
12. Place the spin column into a clean microcentrifuge tube, then add 30-100 µl of Elution Buffer or sterile, distilled water (pH > 7.0) directly into the centre of the column matrix. For higher yield, preheat the Elution Buffer or water to 65 °C). Allow the column to stand at room temperature for 1 minute. Centrifuge the column at 10,000 × g for 1 minute to elute the DNA. The isolated plasmid DNA is ready to use or can be stored at -20 °C.

Notes:

- Carry out all centrifugation steps at room temperature.
- Prior to use, check the Lysis Buffer solution is cloudy or not. If it is cloudy, heat the bottle in a water bath set to 37 °C to ensure all contents are completely dissolve it. Tighten the cap immediately after use to avoid pH change.
- The Maximum DNA yield by this kit is 40 µg. If plasmid DNA yield is low, increase the volume of bacterial culture.
- Use the volume of Working Resuspension Buffer, Lysis Buffer and Neutralization Buffer as suggested above, as too much cell culture can result in incomplete lysis, which will affect plasmid DNA yield and purity.
- 5 ml of LB media is equivalent to 1 rxn.

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