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LiPure™ Plasmid Mini Kit

Cat. #: M0037 Size: 50 rxns/200 rxns

Introduction

LiPure™ Plasmid Mini Kit is designed to isolate up to 30 µg of high-quality plasmid DNA from 1-6 ml bacterial cultures in less than 30 minutes. Plasmid DNA purification follows the alkaline-lysis method and is simplified with Spin Column technology into three quick steps: Bind, Wash, and Elute. Purified plasmid DNA is immediately ready for a wide variety of downstream applications such as routine screening, restriction enzyme digestion, transformation, PCR and DNA sequencing.

Package Information

Components	M0037-05	M0037-20
Buffer P1	15 ml	60 ml
Buffer P2	15 ml	60 ml
Buffer N3	20 ml	80 ml
Buffer PW*	15 ml	2×25 ml
Elution buffer	15 ml	25 ml
RNase A (10 mg/ml)	150 µl	600 µl
Mini Column	50	200
2 mL Collection Tube	50	200

*Prior to use, add absolute ethanol to Buffer PW according to the bottle label.

Storage

RNase A store at -20°C, others at room temperature

Preparation before the experiment and important notes

1. Centrifuge briefly the tube containing RNase A, and transfer it to Buffer P1, mix and store at 2-8°C.

2. Buffer PW is supplied as concentrates. Before using for the first time, add the appropriate amount of ethanol (96-100%) as indicated on the bottle to obtain a working solution.

Protocol

1. Collect 1-6 ml bacterial cultures, centrifuge at 10,000 × g for 1 min. Aspirate and discard the culture media.

Note: For high copy number plasmids, using 1-6 ml bacterial cultures; for low copy number plasmids, using 10 ml bacterial cultures.

2. Add 250 µl Buffer P1/RNase A. Vortex or pipet up and down to mix thoroughly. Complete resuspension of cell pellet is vital for obtaining good yields.

Note: RNase A must be added to Buffer P1 before use.

3. Transfer suspension into a new 1.5 ml microcentrifuge tube.

4. Add 250 µl Buffer P2. Invert and gently rotate the tube several times to obtain a clear lysate. A 2-3 minute incubation may be necessary.

Note: Avoid vigorous mixing as this will shear chromosomal DNA and lower plasmid purity. Do not allow the lysis reaction to proceed more than 5 minutes.

5. Add 350 µl Buffer N3. Immediately invert several times until a flocculent white precipitate forms.

Note: It is vital that the solution is mixed thoroughly and immediately after the addition of Buffer N3 to avoid localized precipitation.

6. Centrifuge at 13,000 x g for 2 min at RT.

7. Insert a DNA Mini Column into a 2 ml Collection Tube. Carefully transfer the cleared supernatant from step 6 to the DNA Mini Column, then centrifuge at 10,000 x g for 1 min at RT.

8. Discard the filtrate and reuse the collection tube. Add 600 µl of Buffer PW to the DNA Mini Column, then centrifuge at 10,000 x g for 1 min at RT.

Note: Buffer PW must be diluted with absolute ethanol according to the bottle label before use.

9. Repeat step 8 for a second wash step.

10. Discard the filtrate and reuse the collection tube. Centrifuge the empty DNA Mini Column at 12,000 x g for 3 min.

Note: This step is critical for removing of trace ethanol that may interfere with downstream applications.

11. Transfer the DNA Mini Column into a clean 1.5 ml microcentrifuge tube, add 30-100 µl Elution Buffer directly to the center of column membrane. Let sit at RT for 2 min, then centrifuge at 10,000 x g for 1 min.

Note: To improve the yield, repeat this step for a second elution step.

12. Discard the column and store the DNA at -20ºC.

Troubleshooting

Low yield

1. Low copy-number plasmid used: Such plasmids may yield as little as 0.1 µg DNA from a 1 ml overnight culture.

2. Culture is overgrown or not fresh: Do not incubate cultures for more than 16 hours at 37ºC. Storage of cultures for extended periods prior to plasmid isolation is detrimental.

3. Poor cell lysis: After addition of Buffer P1/RNase A, vortex to completely resuspend the cells.

4. Poor elution: Increase the volume of elution buffer and incubation time. Repeat the elution.

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Genomic DNA contamination

1. Over mixing of cell lysate upon addition of Buffer AP2: Do not vortex or mix aggressively after adding Buffer AP2.

2. Culture overgrown: Overgrown cultures contain lysed cells and degraded DNA. Do not grow cell longer than 16 hours.