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EasySC Plasmid Mini Purification Kit

Catalog Number: D100-1, D100-2

Table 1. Kit Components and Storage

Kit Component	D100-1 (50 preps)	D100-2 (200 preps)	Storage	Stability
Buffer P1	15 mL	60 mL	RT	The product is stable for one year when stored as directed.
Buffer P2	15 mL	60 mL	RT	
Buffer N3	20 mL	80 mL	RT	
Buffer PW*	15 mL	2×25 mL	RT	
Elution buffer	15 mL	25 mL	RT	
RNase A (10 mg/mL)	150 µL	600 µL	-20 °C	
Mini Column	50	200	RT	
2 mL Collection Tube	50	200	RT	

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

Product Description

EasySC Plasmid Mini Purification 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.

Features

- ❖ Rapid – Purification of plasmid DNA in less than 30 minutes.
- ❖ Safe – No Phenol/chloroform extractions.
- ❖ High-quality – DNA is suitable for a variety of downstream applications.

Things to do before starting

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

Purification 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

Problem	Possible cause and suggestions
Low yield	• Low copy-number plasmid used: Such plasmids may yield as little as 0.1 µg DNA from a 1 mL overnight culture. • 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. • Poor cell lysis: After addition of Buffer P1/RNase A, vortex to completely resuspend the cells. • Poor elution: Increase the volume of elution buffer and incubation time. Repeat the elution.
Genomic DNA contamination	• Over mixing of cell lysate upon addition of Buffer AP2: Do not vortex or mix aggressively after adding Buffer AP2. • Culture overgrown: Overgrown cultures contain lysed cells and degraded DNA. Do not grow cell longer than 16 hours.