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EasyMag Plasmid Mini Purification Kit

Catalog Number: D101-1, D101-2

Table 1. Kit Components and Storage

Kit Component	D101-1 (50 preps)	D101-2 (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	
MagBinding Beads	2.5 mL	10 mL	2-8 °C	

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

Product Description

EasyMag 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 magnetic beads 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. Carefully transfer the cleared supernatant to a new 2 mL microcentrifuge tube. Add 50 µL MagBinding Beads, and vortex thoroughly for 15-20 seconds.
8. Incubate with shaking at RT for 5 min.
9. Transfer the tube to a magnetic rack for an additional 2 min or until beads pellet and supernatant is cleared. With the tube on the magnetic rack remove the supernatant and discard.
10. Add 600 µL of Buffer PW to the tube. Resuspend the beads by pipetting up and down or vortexing at 1,500 rpm for 30 seconds. Replace the tube on the magnetic rack for 2 min or until beads pellet. Remove and discard the supernatant.

    Note: Buffer PW must be diluted with absolute ethanol according to the bottle label before use.
11. Repeat step 10 for a second wash step.
12. Leave the tube on the magnetic rack, open the cap and air dry the beads at RT for 10-15 min.

    Note: This step is critical for removing of trace ethanol that may interfere with downstream applications.
13. Add 30-100 µL Elution Buffer to the tube, resuspend the beads by vortexing. Incubate at 55ºC for 5 min, vortex for 15 seconds every 1-2 min for 5 min.
14. Replace the tube on the magnetic rack for 2 min or until beads pellet. Transfer the cleared supernatant to a clean tube.
15. Store the plasmid 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.