AFTSpin Plasmid Mini Kit

Cat. No. : RK30101

Product components

Components	Component number	Size-1 50 RXN	Size-2 200 RXN
RNase A	RM30533	30 μL	120 μL
Buffer S1	RM30534	15 mL	60 mL
Buffer S2	RM30535	15 mL	60 mL
Buffer S3	RM30536	21 mL	85 mL
Buffer W1	RM30537	28 mL	115 mL
Buffer W2*	RM30538	24 mL	2 × 54 mL
Buffer EB	RM30539	5 mL	20 mL
Spin Column 1	RM30540	50 pk	4 × 50 pk
2 mL Centrifuge Tubes	RM30541	50 pk	4 × 50 pk
1.5 mL Centrifuge Tubes	RM30542	50 pk	4 × 50 pk

*Note: For 50 RXN Buffer W2, add 56 mL of absolute ethanol before use; for 200 RXN Buffer W2, add 126 mL of absolute ethanol to each bottle before use.

Product Description

This kit employs an improved SDS-alkaline lysis method to lyse cells. The silica membrane within the spin column selectively binds plasmid DNA in the solution under high-salt and low-pH conditions. Impurities and other bacterial components are then removed using a washing buffer. Finally, pure plasmid DNA is eluted from the silica membrane using an elution buffer with low salt and high pH. This kit is suitable for extracting up to 20 μg of high-purity plasmid DNA from 1-4 mL of bacterial culture, and the extracted DNA is ready for use in various molecular biology experiments, including restriction enzyme digestion, transformation, PCR, in vitro transcription, and sequencing.

Storage

1. This kit is stable for 12 months when stored at room temperature.
2. RNase A can be stored at room temperature for up to six months, and for long-term storage, it should be stored at -20℃.

Precautions

1. Buffer S2, Buffer S3, and Buffer W1 contain irritating compounds. When handling these buffers, wear latex gloves and safety goggles to avoid contact with skin, eyes, and clothing, and prevent inhalation. If contact with skin or eyes occurs, immediately rinse with plenty of water or saline. Seek medical advice if necessary.
2. After each use, tightly close the reagent bottles promptly to avoid prolonged exposure to air, which may cause volatilization, oxidation, or changes in pH.
3. If precipitation occurs in the solutions stored at 2-8℃, allow the solutions in the kit to stand at room temperature for a while before use. If necessary, preheat the solutions in a 37℃ water bath for 10 minutes to dissolve the precipitates.

Operational Instructions

Reagent Preparation

1. Prior to the first use, add specified amount of absolute ethanol (self-prepared by the user) to Buffer W2 as indicated and mix thoroughly. After adding, clearly mark the reagent bottle to indicate that ethanol has been added to avoid multiple additions.
2. Prior to the first use, add all the RNase A (50 mg/mL) provided in the kit to Buffer S1 (final concentration 100 μg/mL), and then store it at 2 - 8°C. If the RNase A in Buffer S1 is inactivated, there may be a trace amount of RNA remaining in the

extracted plasmid. In this case, simply add additional RNase A (ABclonal, RM29870) to Buffer S1.

User Protocol

1. Take 1 - 4 mL of the bacterial liquid cultured overnight in LB medium (if using a rich medium, the volume of the bacterial liquid should be halved or less). Centrifuge at 12,000 × g for 1 min, discard the supernatant as completely as possible, and collect the bacterial cells.
2. Add 250 µL of **Buffer S1** (please check first whether RNase A has been added) to resuspend the bacterial cell pellet, and vortex until it is completely suspended.

   Note: Incompletely mixed bacterial lumps will affect lysis and result in low extraction yield and purity. If there is no vortex mixer, you can gently flick the bottom of the tube with your finger to break up the precipitate.
3. Add 250 µL of **Buffer S2**, gently and thoroughly invert the tube 4 - 6 times to mix well and lyse the bacteria completely until a clear solution is formed. This step should not exceed 5 min.

   Note: Immediately tighten the bottle cap of Buffer S2 after use to prevent CO_2 in the air from neutralizing the NaOH in Buffer S2 and reducing the bacteriolytic efficiency.

   Note: Avoid vigorous shaking, otherwise it will lead to contamination by genomic DNA.
4. Add 350 µL of **Buffer S3**, and immediately invert the tube gently but thoroughly 6 - 8 times to mix well. Then centrifuge at 12,000 × g for 10 minutes.

   Note: Avoid vigorous shaking, otherwise it will lead to contamination by genomic DNA.

Steps 5 - 7 offer the option of purifying plasmid DNA using either vacuum filtration or centrifugation.

A. Vacuum Filtration Method

5A. Insert the adsorption column SC1 into the interface of the vacuum apparatus. Aspirate the supernatant from Step 4 and transfer it to the adsorption column SC1. Turn on the vacuum and adjust the pressure to -25 to -30 inches of mercury (Hg) to slowly draw off the solution from the tube.

6A. Add 500 µL of **Buffer W1** and aspirate the solution from the tube.

7A. Add 700 µL of **Buffer W2** (please check if absolute ethanol has been added). Aspirate the solution from the tube. Wash again with 700 µL of **Buffer W2** using the same method.

8A. Place the adsorption column SC1 into a 2 mL centrifuge tube and centrifuge at 12,000 × g for 1 minute.

B. Centrifugation Method

5B. Transfer the supernatant from Step 4 to the adsorption column SC1 (placed in a 2 mL centrifuge tube). Centrifuge at 12,000 × g for 1 minute and discard the filtrate.

6B. Place the adsorption column SC1 back into the centrifuge tube, add 500 µL of **Buffer W1**, centrifuge at 12,000 × g for 1 minute, and discard the filtrate.

7B. Place the adsorption column SC1 back into the centrifuge tube, add 700 µL of **Buffer W2** (please check if absolute ethanol has been added), centrifuge at 12,000 × g for 1 minute, and discard the filtrate. Wash again with 700 µL of **Buffer W2** using the same method and discard the filtrate.

8B. Place the adsorption column SC1 back into the 2 mL centrifuge tube and centrifuge at 12,000 × g for 1 minute.

9. Take out the adsorption column SC1 and place it in a new 1.5-mL centrifuge tube. Add 60 - 80 µL of **Buffer EB** to the center of the adsorption column membrane. Let it stand at room temperature for 1 minute, then centrifuge at 12,000 × g for 1 minute.

   Note: To improve the elution efficiency, Buffer EB can be pre-heated in a water bath at 65 - 70°C.
10. Store the obtained DNA eluate at -20°C or use it directly for subsequent experiments.