AFTSpin EndoFree Plasmid Midi Kit

Cat. No. : RK30105

Product components

Components	Component number	Size 50 RXN	Storage
RNase A (10 mg/mL)	RM30219	300 μL	RT
Buffer BL2	RM30211	30 mL	RT
Buffer P1	RM30220	30 mL	RT
Buffer P2	RM30221	30 mL	RT
Buffer P4	RM30214	30 mL	RT
Buffer DW	RM30222	30 mL	RT
Buffer DW2	RM30154	12 mL	RT
Buffer EB2	RM30153	15 mL	RT
Spin Columns 5	RM30197	50 pk	RT
Filtration Columns 1	RM30196	50 pk	RT
2 mL Collection Tubes	RM30188	2 × 50 pk	RT

Product Description

This kit uses a modified SDS-alkaline lysis method to lyse cells. The special Buffer P4 and the Filtration Columns 1 can effectively remove impurities such as endotoxin, protein and other impurities.The kit is suitable for extracting plasmid DNA from 5-15 mL bacterial cultures, and the whole experimental procedure of plasmid DNA extraction could be finished within 1 h. Plasmid DNA prepared is suitable for a variety of downstream applications including restriction enzyme digestion, PCR, sequencing, ligation, transformation and cell transfection.

Storage Conditions

1. This kit is stable for 12 months when stored at room temperature.
2. Buffer P2 may precipitate at low temperature, it can be placed in a water bath at 37℃ for 10 minutes to dissolve precipitate.
3. Prior to the first use, the total volume of RNase A supplied with the kit should be added to Buffer P1 (final concentration 100 μg/mL) and stored at 2-8℃ for 6 months.

Precautions

1. This product is for scientific research use by professionals only and is not intended for clinical diagnosis or treatment.
1. Prior to the first use, add 48 mL of absolute ethanol (self-prepared by the user) to Buffer DW2 and mix thoroughly.
2. Add all the RNase A to Buffer P1 and store at 2-8°. Return to room temperature before use.
3. Check Buffer P2 and Buffer P4 before use to see if there is any precipitate formed. If necessary, dissolve the precipitate by warming at 37°C for several minutes.
4. Unless otherwise specified, all centrifugation steps should be completed at room temperature, using a benchtop centrifuge at a speed greater than or equal to 12,000 rpm (~13,400 x g).
5. The final amount of extracted plasmid is determined by bacterial culture concentration and plasmid copy number. If the culture contains low-copy plasmids or large plasmids (>10 kb), the amount of bacterial culture should be appropriately increased. For these larger extraction volumes, the amount of solution P1, P2, P4 should be increased in proportion, while all other steps remain the same.
6. Reagent bottle should be tightly capped immediately after use to avoid volatilization, oxidation and pH changes caused by prolonged exposure to air.

Operational Instructions

1. Column equilibration: place a Spin Column 5 into a collection tube and add 500 μL **Buffer BL2** to Spin Column 5. Centrifuge for 1 min at 12,000 rpm (~13,400×g). Discard the flow-through, and place Spin Column 5 back into the same collection tube (Use the Spin Column as soon as possible after treated with Buffer BL2).
2. Centrifuge 5-15 mL of the bacterial culture at 12,000 rpm (~13,400 x g) for 1 min, discard the supernatant as much as possible, retain bacterial cell pellet.

   Note: To increase yield, add more microbial culture to the initial pellet in the same tube and repeat step 2 until sufficient bacteria are collected. Excess bacteria may result in inadequate subsequent lysis.
3. Resuspend the bacterial pellet with 500 μL of **Buffer P1** and vortex until thoroughly suspended.

   Note: Incompletely resuspended and mixed cell mass will inhibit complete digestion, resulting in decreased extraction yield and purity.
4. Add 500 μL of **Buffer P2**, gently invert the tube up and down 8-10 times to lyse the bacteria thoroughly.

   Note: Mix gently without do not shaking vigorously to avoid genomic DNA cleavage! After 8-10 inversions, the solution should become clear and free from clumps or floccules. The total lysis time shouldn't exceed 5 minutes. If the solution does not become clear, it may indicate that over high density of bacteria may result in an incomplete lysis, and the amount of bacteria should be reduced.
5. Add 500 μL of **Buffer P4** and immediately invert the solution gently up and down 8-10 times. A white flocculent precipitate will appear when thoroughly mixed. Leave for 10 min at room temperature. Centrifuge at 12,000 rpm (~13,400 x g) for 10 minutes and collect the supernatant.

   Note: Mix immediately after adding Buffer P4 to avoid local precipitation of SDS。If there is still small white precipitate in the supernatant, the supernatant can be taken after centrifugation again.
6. Add the supernatant collected in the previous step to the Filtration columns 1 (Place the filter column into a collection tube). Centrifuge at 12,000 rpm (~13,400 × g) for 2 min, and then transfer the filtrate to a clean 2 mL centrifuge tube (self-prepared).
7. Add 0.3 times the volume of isopropanol to the filtrate and mix thoroughly by inversion. Place Spin Column 5 into a Collection Tube. Transfer the no more than 700 μL of solution to the Spin Column 5 and centrifuge at 12,000 rpm (~13,400 x g) for 1 minute, then discard the waste in the collection tube. Repeat the above operation until the full volume of mixed solution has passed through the spin column.
8. Add 500 μL of **Buffer DW**, centrifuge at 12,000 rpm (~13,400 x g) for 30 seconds, then discard the waste.
9. Add 500 μL of **Buffer DW2 (Confirm that 48 mL absolute ethanol has been added)**, centrifuge at 12,000 rpm (~13,400 x g) for 30 seconds, then discard the waste.
10. Repeat step 9.
11. Place the Spin Column 5 into a collection tube and centrifuge at 12,000 rpm (~13,400 x g) for 3 minutes to remove Buffer DW2 as much as possible, to avoid inhibiting downstream reactions by ethanol from Buffer DW2.
12. Place the Spin Column 5 into a clean centrifuge tube, add 100-300 μL of **Buffer EB2** (preferably heated in a 65-70°C water bath in advance) **to the center of the spin column membrane**, allow to stand at room temperature for 1 minutes, then centrifuge at 12,000 rpm (~13,400 x g) for 1 minutes. If a higher concentration of plasmids is required, the initial elution can be re-added to the membrane of Spin Column 5, allowed to stand at room temperature for an additional 1 minutes, and centrifuged at 12,000 rpm (~13,400 x g) for 1 minute.

    Note: The larger the elution volume, the higher the elution efficiency. Elution volumes below 100 μL should be avoided because such small volume reduces elution efficiency and plasmid yield. Elution with water is also feasible if the pH of the water used is greater than pH 7.5. Maximum elution efficiency occurs between pH 7.0 and 8.5.
13. The obtained DNA should be stored at -20°C or used directly for subsequent experiments.