AFTSpin EndoFree Plasmid Midi Kit

Cat. No. : RK30105

Product components

Components	Component number	Size 50 RXN
Buffer BL2	RM30468	25 mL
Buffer S1	RM30469	30 mL
Buffer S2	RM30470	30 mL
Buffer S3	RM30471	30 mL
Buffer ToxOut	RM30472	30 mL
Buffer W2	RM30473	15 mL
Buffer EB2	RM30474	15 mL
RNase A (10 mg/mL)	RM30475	300 uL
Spin Columns 5	RM30476	50 pk
Collection Tubes	RM30477	50 pk

Product Description

This kit is suitable for extracting plasmid DNA from 5-12 mL bacterial cultures. After the bacterial cells are treated with alkaline lysis, high salt, and low pH conditions, the plasmid is released and specifically and efficiently adsorbed by the silica gel membrane of the centrifugal column. The bacterial endotoxins can be removed by washing with Buffer ToxOut. Then, proteins and other impurities can be removed by washing with Buffer W2. Finally, high - purity endotoxin - free plasmid DNA is eluted under low - salt and high - pH conditions. The purified plasmid DNA is suitable for various routine operations, including conventional cell transfection, restriction enzyme digestion, PCR, sequencing, ligation, transformation, library screening, in vitro translation, etc.

Storage Conditions

1. This kit is stable for 12 months when stored at room temperature.
2. **Prior to the first use, the total volume of RNase A supplied with the kit should be added to Buffer S1 and stored at 2-8°C.**

Precautions

1. RNase A is provided in the kit. After brief centrifugation, add all the RNase A to Buffer S1, mix well, and store at 2 - 8°C. Before use, let it stand at room temperature for a while until it returns to room temperature.
2. **Before the first use, add 45 mL of absolute ethanol to Buffer W2. After use, immediately tighten the bottle cap to prevent the evaporation of ethanol.**
3. Each time before use, check whether precipitates have formed in Buffer S2 and Buffer S3. If there are precipitates, dissolve them at 37°C before use.
4. The general incubation time for bacteria is 12 - 16 hours. If the inoculum size is large, the incubation time should be reduced. Over - cultivation may reduce the quality of the plasmid and even lead to plasmid DNA mutation.
5. The yield of the plasmid is closely related to the amount of bacteria, plasmid copy number, plasmid size, and the degree of standardized operation.
6. If the plasmid to be extracted is a low - copy plasmid, a large plasmid larger than 10 kb, an Agrobacterium plasmid, or a Gram - positive bacterium plasmid, increase the amount of bacterial cells. Use 5 - 12 mL of an overnight culture. At the same time, increase the amounts of Buffer S1, Buffer S2, and Buffer S3 proportionally. Pre - heat the elution buffe in a 60°C water bath. Appropriately extend the adsorption and elution time to increase the extraction efficiency. The other steps remain the same.

Operational Instructions

1. Column equilibration: Add 400 μL of **Buffer BL2** to Spin Columns 5 (place the adsorption column in the collection tube), centrifuge at 12,000 rpm (~13,400 × g) for 1 min, discard the filtrate in the collection tube, and put the adsorption column back into the collection tube.

   Note: Try to use the column treated with the eBuffer BL2 on the same day to avoid affecting the subsequent experimental results due to long - term storage.

2. Take 5 - 12 mL of an overnight - cultured bacterial liquid, centrifuge at 12,000 rpm (~13,400 × g) for 1 min at room temperature, and remove the supernatant as completely as possible.

   Note: If there is a large amount of bacterial liquid, it can be added to the tube in batches for centrifugation to collect the bacterial cells. Do not use too much bacterial cells, otherwise the lysis will be insufficient.

3. Add 500 μL of **Buffer S1** (please check if RNase A has been added first), and then vortex or pipette thoroughly to resuspend the bacterial pellet evenly.

   Note: The bacterial c ell pellet must be suspended evenly. If there are incompletely suspended bacterial lumps, it will affect the lysis, resulting in a decrease in the concentration and purity of the extracted plasmid.

4. Add 500 μL of **Buffer S2**, gently invert and mix to completely lyse the bacterial cells until the solution becomes clear and viscous.

   Note: Invert and mix gently, do not shake vigorously to avoid breaking the genomic DNA. At this time, the bacterial liquid should become clear and viscous, and the time used should not exceed 5 min to prevent the plasmid from being damaged. If the solution does not become clear, it may indicate that the amount of bacteria is too large. You can increase the amount of Buffer S2, and the amount of Buffer S3 should also be increased accordingly in the subsequent operations.

5. Add 500 μL of **Buffer S3**, and immediately invert gently to mix. A red-yellow precipitate will form. Continue mixing until the solution turns completely yellow. Let it stand at room temperature for 2 minutes, then centrifuge at 12,000 rpm (~13,400 × g) for 5 minutes.

   Note: After adding Buffer S3, mix immediately to avoid local precipitation. If there are still purple floating substances in the supernatant, it means that the renaturation is insufficient. Continue mixing until the solution completely turns into a clear yellow color.

6. Carefully transfer the supernatant to a new centrifuge tube. Add 0.3 times the volume of isopropanol and mix. Then transfer the solution to the adsorption column and let it stand for 2 minutes to allow the plasmid DNA to bind fully with the silica membrane in the column. Centrifuge at 12,000 rpm (~13,400 × g) for 30 s, and discard the filtrate in the collection tube.

   Note: The maximum volume of the adsorption column is 750 μL. The remaining liquid should be passed through the column in batches.

7. Add 500 μL of **Buffer ToxOut** to the adsorption column, let it stand at room temperature for 5 min, and centrifuge at 12,000 rpm (~13,400 × g) for 1 min. Discard the filtrate in the collection tube.

8. Add 600 μL of **Buffer W2** (please check if absolute ethanol has been added first), centrifuge at 12,000 rpm (~13,400 × g) for 30 s at room temperature, and discard the filtrate in the collection tube.

   Note: Buffer W2 is a concentrated solution. Add absolute ethanol as required. After use, immediately tighten the bottle cap to prevent the evaporation of alcohol.

9. Add 500 μL of **Buffer W2**, and centrifuge at 12,000 rpm (~13,400 × g) for 2 min at room temperature to remove the residual liquid.

   Note: This step cannot be omitted, otherwise the residual ethanol will affect the downstream experiments.

10. Place the adsorption column in a new 1.5 mL centrifuge tube (prepared by yourself), add 100 - 300 μL of elution buffer **Buffer EB2** to the center of the adsorption membrane, let it stand at room temperature for 2 min, and centrifuge at 12,000 rpm (~13,400 × g) for 1 min to collect the plasmid solution in the centrifuge tube.

    Note: To increase the elution efficiency, Buffer EB2 can be pre - heated at 60°C. Buffer EB has a single component and will not affect downstream restriction enzyme digestion, transfection, or other molecular biology experiments. Please use it with confidence. Elution with

deionized water is not recommended. If it is necessary to use deionized water for elution, adjust its pH value to between 8.0 - 8.5 with NaOH before use. To increase the plasmid recovery rate, the obtained solution can be added back to the centrifuge tube, let it stand at room temperature for 2 min, and centrifuge again for collection.

11. The obtained DNA should be stored at -20°C or used directly for subsequent experiments.