AFTSpin EndoFree Plasmid Maxi Kit

Cat. No. : RK30103

Product components

Components	Component number	Size 10 RXN	Storage
RNase A (10 mg/mL)	RM30107	750 μL	4℃
Buffer ER	RM30108	25 mL	4℃
Buffer P1	RM30103	75 mL	4℃
Spin Column 3	RM30182	10 pk	RT
Collection Tube 50 mL	RM30191	4 × 5 pk	RT
Buffer P2	RM30104	75 mL	RT
Buffer N3	RM30106	75 mL	RT
Buffer PR*	RM30109	65 mL	RT
Buffer WB**	RM30110	2 × 25 mL	RT
Buffer EB	RM30111	20 mL	RT

*Note: Add 38 mL of absolute ethanol to Buffer PR prior to initial use.

**Note: Add 100 mL of absolute ethanol to each Buffer WB bottle prior to initial use. (Final reagent volume in each Buffer WB bottle is 125 mL.)

Product Description

This kit uses a modified SDS-alkaline lysis method to lyse cells. The unique endotoxin remover can effectively remove endotoxins. The silicone matrix membrane in the spin column selectively binds plasmid DNA at high salt and low pH conditions, allowing for impurities and other bacterial components to be removed by the addition of Buffer PR and Buffer WB. The purified plasmid DNA is eluted from the silicone matrix membrane using low-salt and high-pH Buffer EB.

The yield and quality of extracted plasmids are dependent upon the species and culture conditions of host bacteria, cell lysis, plasmid copy number, plasmid stability, and use of antibiotics.

Storage

1. This kit is stable for 12 months when stored at room temperature.
2. RNase A, Buffer ER and Buffer P1 can be transported at room temperature for a short time, but for long-term storage, RNase A and Buffer ER should be kept at -20℃; Buffer P1 should be kept at 4℃.
3. Minor precipitation of reagents does not affect experimental results. If necessary, reagents can be placed in a water bath at 37℃ for 10 minutes to dissolve precipitate.

Highlights

1. The unique Deproteinization Buffer (Buffer PR) efficiently removes residual nucleases, including those from host strains with abundant nucleases such as JM series and HB101, effectively preventing plasmid degradation by nucleases.
2. Neither toxic reagents (i.e. phenol and chloroform) nor ethanol precipitation are required.
3. Final products have minimal endotoxin content (< 0.1 EU/μg DNA).
4. It is a fast and convenient high-quality plasmid DNA extraction method. From 150-300 mL of LB medium, 0.5-2 mg of pure high-copy plasmid DNA can be rapidly extracted with an extraction efficiency of 80-90%.

Scope of Application

The plasmid DNA extracted by this kit can be directly used in cell transfection experiments along with various molecular biological experiments such as enzymatic digestion, transformation, PCR, in vitro transcription, and sequencing.

Precautions

1. This product is for scientific research use by professionals only and is not intended for clinical diagnosis or treatment.
2. Please wear a lab coat and disposable gloves for your safety and health.
3. Unless otherwise specified, all centrifugation steps should be completed at room temperature, using a benchtop centrifuge at a speed at 8,000 rpm (~8,200 x g).
4. 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, N3 should be increased in proportion, while all other steps remain the same.
5. Buffer EB does not contain EDTA as a chelating agent and does not affect downstream digestion, ligation, and other reactions. 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. Elution with water should ensure that the pH of the water used is within this range as low elution may occur if the pH is too low. During elution, sterilized distilled water or Elution Buffer can be heated to 60℃ before use to increase elution efficiency. Plasmids eluted with water should be stored at -20℃. Plasmid DNA may be eluted with TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0) if long-term storage is required, but EDTA may affect downstream digestion reactions and should be diluted appropriately when used.
6. At low temperatures, SDS in Buffer P2 may produce turbidity or precipitation. In this case, the solution may be clarified by heating in a 37℃ water bath for a few minutes, without vigorous shaking to avoid excessive foam formation.
7. Reagent bottle should be tightly capped immediately after use to avoid volatilization, oxidation and pH changes caused by prolonged exposure to air.

Operational Instructions

Reagent Preparation

1. Prior to the first use, add specified amount of absolute ethanol (self-prepared by the user) to Buffer PR and Buffer WB 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 supplied with the kit to Buffer P1 (final concentration is 100 μg/mL) and store at 2-8℃. If RNase A becomes inactivated in Buffer P1, trace RNA residues may be present in the extracted plasmid; addition of fresh RNase A to Buffer P1 is sufficient to restore the reagent (or alternatively purchased or self-prepared).

User Protocol

1. Centrifuge 150-200 mL (up to a maximum of 300 mL) of the bacterial culture at 8,000 rpm(~8,200 x g) for 1 minute, decant the supernatant as much as possible, and retain the bacterial cell pellet.

   Note: Repeatedly add 50 mL bacterial culture to the same 50 mL centrifuge tube with the initial pellet and repeat step 1 until sufficient bacterial cell pellet has been collected.

2. Resuspend the bacterial pellet with 7.5 mL of **Buffer P1** (**confirm that RNase A has been added**) and pipette up and down or vortex until thoroughly suspended.

   Note: Incompletely resuspended and mixed cell mass will inhibit complete digestion, resulting in decreased extraction yield and purity.

3. Add 7.5 mL **Buffer P2**, gently invert the centrifuge tube up and down 6-8 times to fully mix and allow to stand at room temperature for 4-5 minutes.

   Note: Mix gently without do not shaking vigorously to avoid genomic DNA cleavage! After 6-8 inversions, the solution should become clear and free from clumps or floccules. If the bacteria are not completely dispersed, or a small number of clumps or floccules are evident, the solution can be inverted for another 3-5 times and allowed to stand at room temperature for 2-3 minutes, with the total lysis time not exceeding 5 minutes.

4. Add 7.5 mL of **Buffer N3** and immediately invert the solution gently up and down 6-8 times. White flocculent precipitate will appear when thoroughly mixed. Centrifuge at 8,000 rpm(~8,200 x g) for 10-15 minutes, carefully remove the supernatant to a new tube and avoid pipetting up the floating white precipitate.

   Note: Mix immediately after adding Buffer N3 to avoid local precipitation of SDS. Avoid shaking or excessive inversions, otherwise the quality of the resulting plasmids will be easily decreased.

5. Add 0.1 times the volume of the supernatant (10%, approximately 2.4 mL) of **Buffer ER** to the sample, invert and rotate the 50 mL tube to mix well.

(Step 6-8 are optional. If the subsequent experiment is sensitive to endotoxin, follow step 5-7 to further improve the efficiency of endotoxin removal.)

6. After adding the endotoxin removal reagent Buffer ER and mixing well, place the tube in an ice bath or insert it into crushed ice (or the freezer compartment of a refrigerator) for 5 minutes, with occasionally mixing, until the solution becomes clear and transparent (or still slightly turbid).

   Note: When Buffer ER is added to the supernatant, the supernatant will become turbid but should return to clear (or slightly turbid) after ice bath.

7. Allow to stand at room temperature for 3-5 minutes until the solution becomes turbid. Invert to mix well.

   Note: If the room temperature is low, or you want to accelerate the reaction, the solution can be placed in a water bath at 37-42°C to quickly become turbid.

8. Centrifuge at 8,000 rpm(~8,200 x g) for 10 minutes at room temperature for phase separation. The upper aqueous phase contains DNA and the lower blue oily phase contains endotoxin and other impurities. Transfer the upper aqueous phase containing DNA to a new tube (take care not to pipette up the blue oily layer) and discard the oily layer.

   Note: The temperature during the centrifugation process should be above 25°C. If the temperature is too low, effective phase separation cannot be achieved. If inadequate phase separation is observed, the centrifugation time may be extended to 15 minutes.

9. Add 0.5 times the volume of isopropanol to the upper aqueous phase and mix thoroughly by inversion.Place **Spin Column 3** into a Collection Tube. Transfer the no more than 10 mL of solution to the spin column membrane and centrifuge at 8,000 rpm(~8,200 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.

   Note: Due to the large tilt angle of centrifuge rotors, it is not recommended to add more than 10 mL to the spin column each time to prevent liquid leakage.

10. **Optional step**: Add 10 mL of **Buffer PR** (**confirm that absolute ethanol has been added**), centrifuge at 8,000 rpm(~8,200 x g) for 1 minute, then discard the waste.

    Note: This step removes impurities such as trace nucleases. For host strains that are EndA+ strains such as JM series and HB101, or for wild-type strains with abundant nucleases, this step is strongly recommended; this step is optional when using deficient strains with low nuclease expression, such as XL-1 Blue, Top10 and DH5α.

11. Add 10 mL of **Buffer WB** (**confirm that absolute ethanol has been added**), centrifuge at 8,000 rpm(~8,200 x g) for 1 minute, then discard the waste. Repeat with an additional 10 mL of Buffer WB.
12. Place the **Spin Column 3** into an empty Collection Tube and centrifuge at a maximum speed of 8,000 rpm(~8,200 x g) for 3 minutes to remove any residual ethanol from the matrix membrane. Open the tube cap and allow to fully dry at room temperature for 2-3 minutes. Residual ethanol on the column can inhibit downstream reactions and severely reduce elution efficiency and plasmid yield.
13. Move the **Spin Column 3** to a clean centrifuge tube and add 1-2 mL of **Buffer EB** (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 2 minutes, then centrifuge at 8,000 rpm(~8,200 x g) for 1 minute. To increase the efficiency of plasmid recovery, the resulting elution can be re-added to Spin Column 3, allowed to stand at room temperature for 1 minute and centrifuged at 8,000 rpm(~8,200 x g) for 1 minute.

Note: The larger the elution volume, the higher the elution efficiency. Elution volumes below 1 mL should be avoided as too small volume reduces elution efficiency and plasmid yield.

14. The resulting DNA eluate should be stored at -20℃ or used directly for subsequent experiments.