AFTSpin EndoFree Plasmid Mini Kit

Cat. No. : RK30102

Product components

Components	Component number	Size 50 RXN	Storage
RNase A (10 mg/mL)	RM30107	125 μL	4℃
Buffer ER	RM30108	5 mL	4℃
Buffer P1*	RM30103	12.5 mL	4℃
Buffer BL**	RM30101	5 mL	RT
Spin Column 2	RM30181	50 pk	RT
Collection Tube (2 mL)	RM30190	50 pk	RT
Buffer P2	RM30104	12.5 mL	RT
Buffer N3	RM30106	12.5 mL	RT
Buffer PR***	RM30109	16 mL	RT
Buffer WB****	RM30110	13 mL	RT
Buffer EB	RM30111	10 mL	RT

*** Note: Prior initial use, add the full volume of supplied RNase A to Buffer P1 (final concentration: 100 μg/mL) and store at 2-8℃.**

**** Note: Equilibration Buffer (Buffer BL) is a strong alkaline solution; if accidentally touched, please wash with a large amount of tap water. Tightly seal the bottle after use to limit contact with air. Store at room temperature. Precipitation may occur during storage. If present, heat the solution to 37℃ until the precipitate disappears completely.**

***** Note: Add 10 mL of absolute ethanol to Deproteinization Buffer (Buffer PR) prior to initial use.**

****** Note: Add 52 mL of absolute ethanol to Wash Buffer (Buffer WB) bottle prior to initial use.**

Product Description

This kit uses a modified SDS-alkaline lysis method to lyse cells. Crude extract is selectively bound to the spin column by a unique endotoxin scavenger and centrifuged to remove endotoxin. 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.

Product Features

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) and excellent cell transfection.
4. The plasmids obtained have high yield and purity and can be directly used in enzyme digestion, transformation, PCR, in vitro transcription, sequencing, and other molecular biology experiments. To extract up to 20 μg purified plasmids, transform host bacteria with high copy plasmid, inoculate a single colony in 1.5-4.5 mL LB medium with appropriate antibiotics, and culture overnight for 12-16 hours prior to extraction.

Storage Conditions

1. This kit is stable for 12 months when stored at room temperature.
2. Buffer ER can be transported at room temperature for short-term. It can be stored at 4℃ for 12 months.
3. RNase A is preserved in a ready-to-use glycerol buffer. It can be stored at room temperature of 25℃ for at least 6 months, at 4℃ for 12 months, and for long-term storage, it should be placed at -20℃.

4. After adding RNase A to Buffer P1, it should be stored at 2 - 8℃ and used up within 3 months as soon as possible.
5. Under the storage condition of 2 - 8℃, if the solution forms precipitates, the solution in the kit should be placed at room temperature for a while before use. If necessary, it can be preheated in a 37℃ water bath for 10 minutes to dissolve the precipitates. The occasional presence of particles in the reagents will not affect the experimental results.

Scope of Application

The plasmid DNA extracted by the kit can be applied to various routine operations, including enzyme digestion, PCR, sequencing, ligation, transformation, in vitro translation.

Notes

1. The Equilibration Buffer (Buffer BL) is a strong alkaline solution; Please take appropriate precautions to avoid direct contact with skin.
2. 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).
3. The final amount of extracted plasmid is determined by bacterial culture concentration and plasmid copy number. For high copy plasmids, inoculate single colonies in 1.5-4.5 mL LB medium with appropriate antibiotics and culture overnight for 12-16 hours, allowing for an extracted yield of up to 20 μg pure plasmids. If the culture contains low-copy plasmids or large plasmids (>10 kb), the amount of bacterial culture should be appropriately increased, generally not to exceed more than 10 mL (excessive bacteria will lead to inadequate subsequent lysis). For these larger extraction volumes, the amount of solution P1, P2, N3 should be increased in proportion, while all other steps remain the same.
4. **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.
5. At a low ambient temperature, 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.
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

Preparation before the experiment

1. Prior to the first use, add specified amounts 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 the total volume RNase A supplied with the kit to Buffer P1 (final concentration 100 μg/mL) and store at 2-8°C. If RNase A becomes inactivated in Buffer P1, trace RNA residues may be present in the extracted plasmid; addition of fresh RNase A （ABclonal，RM29870）to Buffer P1 is sufficient to restore the reagent.

Operating Procedures

1. To equilibrate **Spin Column 2**, insert the new **Spin Column 2** into a **Collection Tube** and pipette 100 μL of **Buffer BL** onto the column membrane. Centrifuge at 12,000 rpm (~ 13,400 x g) for 1 minute; discard the waste from the collection tube and return the spin column to the collection tube.

Note: Pretreatment of the spin column with Buffer BL can greatly improve the binding ability of the membrane to nucleic acids, thereby improving the recovery efficiency or yield of the silica column.

2. Centrifuge 1.5-5 mL of the bacterial culture at 12,000 rpm (~13,400 x g) for 30 seconds, pour out the supernatant as much as possible, retaining bacterial cell pellet.

   Note: To increase yield, add more microbial culture to the initial pellet in the same 1.5 mL tube and repeat step 1 until sufficient bacteria are collected. (The amount of bacterial culture used for extraction of high-copy plasmids should generally not exceed 5 mL, and the amount of bacteria used for extraction of low-copy plasmids should generally not exceed 10 mL. Excess bacteria may result in inadequate subsequent lysis).

3. Resuspend the bacterial pellet with 250 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 250 μL of **Buffer P2**, gently invert the 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! The whole process should not take more than 5 minutes to prevent the plasmid from being damaged. At this time, the bacterial liquid should become clear and viscous. If it is very cloudy, it may be due to an excessive amount of bacterial cells and incomplete lysis. In such a case, the amount of bacterial cells should be reduced.

5. Add 250 μL of **Buffer N3** and immediately invert the solution gently up and down 6-8 times. A white flocculent precipitate will appear when thoroughly mixed. Centrifuge at 12,000 rpm (~13,400 x g) for 10 minutes and carefully remove the supernatant.

   Note: Mix immediately after adding Buffer N3 to avoid local precipitation of SDS.

6. Add 0.1 volume (10% of the volume of the supernatant, approximately 75 μL) of Buffer ER to the supernatant obtained in the previous step. Mix by inverting and swirling.

Steps 7 - 9 are optional steps. If the extracted plasmid needs to be transfected downstream into relatively fragile cell lines such as primary cultured cells, the following steps can be followed to further completely remove endotoxins to achieve the best transfection results.

7. Place the supernatant, which has been mixed evenly after adding Buffer ER, in an ice bath (or in a -20°C freezer) for 5 minutes, with occasionally mixing, until the solution becomes clear and transparent (or still slightly turbid).

   Note: After Buffer ER is added to the supernatant, it may become turbid briefly, but it should return to being clear (or slightly turbid) after the ice bath.

8. Let it stand at room temperature for 3 - 5 minutes. As the temperature returns to room temperature, the solution will quickly become turbid. Mix it evenly by inverting.

   Note: If the room temperature is relatively low or you want to speed up the process, you can use a water bath at 37 - 42°C. It will quickly become turbid. Then mix it evenly by inverting.

9. Centrifuge at 12,000 rpm for 10 minutes at room temperature to separate the phases. The upper aqueous phase contains plasmid DNA, while the lower blue oily phase contains endotoxins and other impurities. Transfer the upper aqueous phase containing DNA to a new tube (be careful not to pipette up the blue oily phase, which contains endotoxins and other impurities), and discard the oily phase.

   Note: During the centrifugation process, the temperature needs to be higher than 25°C. If the temperature is too low, effective separation of the phases cannot be achieved. If it is found that the phases cannot be effectively separated, the centrifugation time can be extended to 15 minutes.

10. Add 0.5 volume of isopropanol (about 370 μL) to the supernatant. Mix thoroughly by inverting multiple times. Place **Spin Column 2** into a **Collection Tube**. Transfer the no more than 700 μL of solution to the spin column membrane 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.

11. **Optional step**: Add 500 μL of **Buffer PR**, centrifuge at 12,000 rpm (~13,400 x g) for 30 seconds and 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.

12. Add 600 μL of **Buffer WB (confirm that absolute ethanol has been added)**, centrifuge at 12,000 rpm (~13,400 x g) for 30 seconds, then discard the waste. Add an additional 600 μL of **Buffer WB**, centrifuge at 12,000 rpm (~13,400 x g) for 30 seconds, then discard the waste.
13. Place the **Spin Column 2** into an empty **Collection Tube** and centrifuge at 12,000 rpm (~13,400 x g) for 2 minutes to remove Buffer WB as much as possible, to avoid inhibiting downstream reactions by residual ethanol from Buffer WB.
14. Place the **Spin Column 2** into a clean centrifuge tube, add 50-100 μL of **Buffer EB** (preferably heated in a 80-90°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 12,000 rpm (~13,400 x g) for 1 minutes. If a higher concentration of plasmid DNA is required, the initial elution can be re-added to the membrane of **Spin Column 2**, allowed to stand at room temperature for an additional 2 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 30 μL should be avoided as too small volume reduces elution efficiency and plasmid yield.

15. The resulting DNA eluate should be stored at -20°C or used directly for subsequent experiments.

Determination of DNA concentration and purity

1. The resultant plasmid DNA can be visualized by agarose gel electrophoresis and ultraviolet spectrophotometer for concentration and purity. **An OD260 value of 1 corresponds to approximately 50 μg/mL DNA. Gel electrophoresis may show a single band or ≥2 DNA bands**, which is mainly caused by different migration positions of plasmids with different degrees of supercoiling and is additionally affected by the length of culture growth and handling during extraction.
2. **The exact molecular size of plasmid DNA** must be determined based on the results of agarose gel electrophoresis **after linearization by enzyme digestion**. Plasmids in the circular or supercoiled state cannot be electrophoresed to determine their exact size because of their uncertain migration positions.