AFTSpin Fast Plasmid Mini Kit

Cat. No. : RK30101

Product components

Components	Component number	Size-1 50 RXN	Size-2 200 RXN	Storage
RNase A (10 mg/mL)	RM30107	125 μL	500 μL	-20℃
Buffer P1	RM30103	12.5 mL	50 mL	4℃
Buffer BL	RM30101	5 mL	20 mL	RT
Spin Column 2	RM30181	50 pk	200 pk	RT
Collection Tube 2mL	RM30190	50 pk	200 pk	RT
Buffer P2	RM30104	12.5 mL	50 mL	RT
Buffer P3	RM30105	17.5 mL	70 mL	RT
Buffer PR*	RM30109	16 mL	64 mL	RT
Buffer WB**	RM30110	13 mL	2 × 25 mL	RT
Buffer EB	RM30111	10 mL	20 mL	RT

*Note: Prior to initial use, add 10 mL of absolute ethanol (for size 50 RXN) to Buffer PR; add 40 mL of absolute ethanol (for size 200 RXN) to Buffer PR.

**Note: Prior to initial use, add 52 mL of absolute ethanol (for size 50 RXN) to Buffer WB; Add 100 mL of absolute ethanol (for size 200 RXN) to each Buffer WB bottle (final volume of each Buffer WB bottle is 125 mL).

Product Description

This kit uses a modified SDS-alkaline lysis method to lyse cells. 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 and Buffer P1 can be transported at room temperature for a short time, but for long-term storage, RNase A should be kept at -20℃ and Buffer P1 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. 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-number 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.

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.

Precautions

1. This product is for scientific research use by professionals only and is not intended for clinical diagnosis or treatment.
2. The Equilibration Buffer (Buffer BL) is a strong alkaline solution; wear appropriate PPE and avoid direct contact with skin.
3. 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).
4. The final amount of extracted plasmid is determined by bacterial culture concentration and plasmid copy number. For high-copy-number 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-number 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, P3 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°C before use to increase elution efficiency. Plasmids eluted with water should be stored at -20°C. 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 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°C 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 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 to Buffer P1 is sufficient to restore the reagent (or alternatively purchased or self-prepared).

User Protocol

1. Centrifuge 1.5-4.5 mL of the bacterial culture at 12,000 rpm (~13,400 x g) for 30 seconds, decant the supernatant as much as possible, retaining bacterial cell pellet.

   Note: To increase yield, add more microbial culture to 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).
2. Resuspend the bacterial pellet with 250 μL of **Buffer P1** (**confirm that RNase A has been added**) and vortex until thoroughly suspended.

   Note: Incompletely resuspended and mixed cell mass will inhibit complete digestion, resulting in decreased extraction yield and purity. If a vortexer is unavailable, flick the bottom of the tube with a finger to disperse the bacterial mass evenly though the lysis solution.
3. 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 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 350 μL of **Buffer P3** and immediately invert the solution gently up and down 6-8 times. 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 P3 to avoid local precipitation of SDS.Pretreatment of Spin Column 2 with Equilibration Buffer is a necessary step. See "Step 5" for the protocol.

5. To equilibrate S**pin Column 2**, insert a new Spin Column2 into a Collection Tube and pipette 100 μL of Buffer BL onto the column membrane. Centrifuge at 12,000 rpm for 1 minute; discard the waste from the collection tube and return the spin column to the collection tube.
6. Add the supernatant obtained in the step 4 to a pretreated **Spin Column 2** (the Spin Column should be placed in a collection tube), centrifuge at 12,000 rpm (~13,400 x g) for 30-60 seconds and discard the waste in the collection tube.
7. **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.

8. 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.
9. 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.
10. Place the **Spin Column 2** into a clean centrifuge tube, add 50-100 μL 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 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 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.

11. 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.