艾科瑞生物
Accurate Biology

Instruction for Use

SteadyPure Plasmid DNA Extraction Kit

AG 21001
Version.V3E1

Research Use Only
Not For Diagnosis Procedures

1. Description

This Product is optimized for fast, simple and cost-effective extraction of plasmid DNA. The kit is based on optimized SDS Alkaline Lysis method and highly effective centrifuge absorption columns, to retrieve high quality and high purity plasmid DNA. Retrieved plasmid DNA could be used for further experiments including DNA sequencing analysis, in vitro transcription, transformation, restriction enzyme digestion and other enzymatic reactions.

2. Kit Information

Kit Name	Cat. No	Specification
SteadyPure Plasmid DNA Extraction Kit	AG 21001	50 rxns

3. Transportation and Storage

	Package 2-1	Package 2-2
Storage	Store at –20°C	Store at Room Temperature
Transportation	Transport at –20°C Dry Ice or Blue Ice Condition	Transport at Room Temperature

4. Kit Components

Package 2-1 (-20 °C Storage)

Kit Components	Volume
RNase A (10mg/ml)	140 µl

Package 2-2 (Room Temperature Storage)

Kit Components	
Buffer RS*1	14 ml
Buffer LS	14 ml
Buffer BS	18 ml
Buffer WA	25 ml
Buffer WB*2	27 ml
Elution Buffer	10 ml
Plasmid DNA Mini Columns	50 sets
Collection Tubes	50 pcs

*1: Before first use, add RNase A (10mg/ml) into Buffer RS at a volume ratio of RNase A:Buffer RS=1:100. Mix well and properly mark the container. Buffer RS with RNase A added shall be stored at 2~8 °C for up to 6 months.

*2: Before first use, add 63 ml of 100% ethanol into the Buffer RWB bottle (Ratio of Buffer RWB:100% ethanol = 3:7). Mix well and properly mark the container. Buffer RWB with 100% ethanol added shall be stored at Room Temperature.

5. Not Provided Experimental Materials

100% Ethanol, RNase Free Water, 1.5 ml centrifuge tubes (RNase Free), 2.0 ml centrifuge tubes (RNase Free), water-bath instrument.

6. Recommendation and Experimental Precautions

(1) For precipitant in Buffer LS, heat and dissolve at 37 °C.

(2) To improve elution efficiency of plasmid DNA, recommend to heat the elution buffer or RNase Free Water to 50~65 °C, then use to elute plasmid DNA from the Plasmid DNA Mini Columns.

(3) Precool Buffer BS on ice or at 4°C before use.

(4) After mix of Buffer LS and Buffer BS, do not violently shake and mix. Violent operation might result in genomic DNA

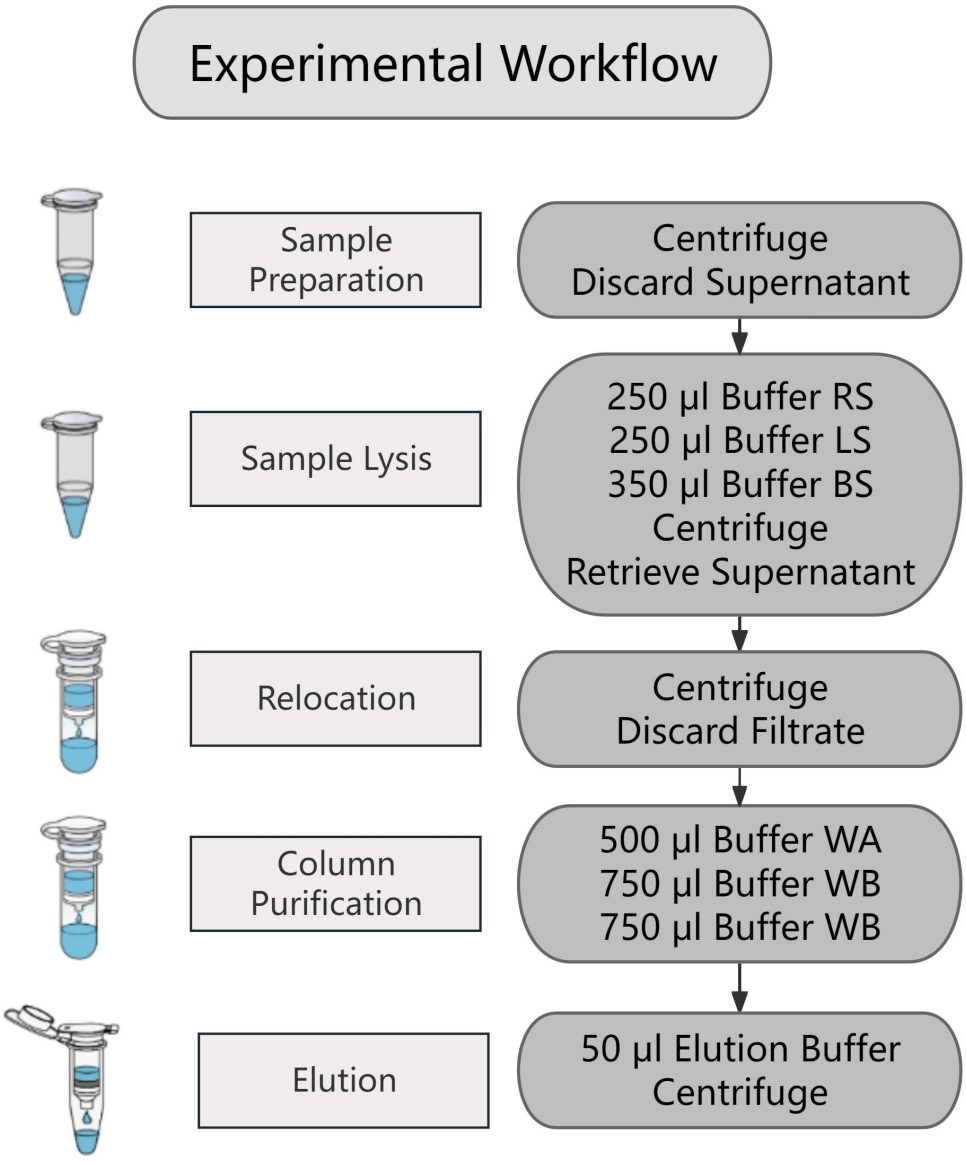
contamination.

(5) After adding of Buffer BS, gently and thoroughly mix to precipitate protein and genomic DNA to form white floccules at bottom. Gradually increase intensity of mixing to form precipitation.

(6) For downstream DNA sequencing analysis of retrieved plasmid DNA, recommend to use RNase Free Water to elute at the last Elution step.

(7) For long term storage of Plasmid DNA, recommend to use and store in Elution Buffer.

(8) For large sample volume, accordingly increase buffer volumes and repeat column purification steps.



7. Protocol

7.1 Sample Preparation

Sample 1~5 ml of overnight cultivated bacteria solution, centrifuge for 2 min at 12,000 rpm, Room Temperature. Discard supernatant.

Note: OD value of bacteria solutions should be within 2~8. For sample with OD>8, proportionally increase reagent use volume.

7.2 Sample Lysis

(1) Add 250 µl of Buffer RS (with RNase A added). Use pipette or vortex to mix well and fully suspend bacteria, until not visible bacteria mass.

(2) Add 250 µl of Buffer LS, gently invert 6~8 times to thoroughly mix until the solution is visually transparent.

(3) Add 250 µl of precooled Buffer BS. White floccules will occur. Gently invert 6~8 times to thoroughly mix.

Note: Total time span of conducting step (2) and (3) should not be more than 5 min.

Note: Keep mixing gentle to avoid release of bacteria genomic DNA to cause contamination.

(4) Settle for 2 min. Centrifuge for 10 min at 12,000 rpm, Room Temperature. Retrieve supernatant for further operation.

7.3 Relocation to column

Relocate retrieved supernatant solution to the Plasmid DNA Mini Columns. Settle for 1 min at Room Temperature, then Centrifuge for 1 min at 12,000 rpm, Room Temperature. Discard the filtrate at bottom tube.

7.4 Column Purification

(1) Add 500 µl of Buffer WA to the Plasmid DNA Mini Columns. Centrifuge for 1 min at 12,000 rpm, Room Temperature. Discard the filtrate at bottom tube.

(2) Add 750 µl of Buffer WB to the Plasmid DNA Mini Columns. Centrifuge for 1 min at 12,000 rpm, Room Temperature. Discard the filtrate at bottom tube.

Note: Please ensure that Buffer WB is already added with 100% ethanol at ratio of Buffer RWB:100% ethanol = 3:7.

(3) Repeat Step (2) Once.

(4) Relocate the Plasmid DNA Mini Columns onto new 2.0 ml Collection tubes. Centrifuge for 2 min at 12,000 rpm, Room Temperature.

Note: Avoid absorption column bottom touching the inner well of bottom tubes, while removing the absorption column.

7.5 Plasmid DNA Elution and Retrieve

(1) Relocate the Plasmid DNA Mini Columns onto new 1.5 ml RNase Free Centrifuge tubes. Carefully add 50 µl of Elution Buffer or RNase Free Water, avoid damaging the membranes. Settle at room temperature for 1 min.

Note: To improve elution efficiency of plasmid DNA, recommend to heat the elution buffer or RNase Free Water to 50~65 °C.

(2) Centrifuge for 1 min at 12,000 rpm, Room Temperature to elute the plasmid DNA.

(3) Retrieve the plasmid DNA at the bottom centrifuge tubes. Discard the columns.

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