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LiPure™ Endotoxin-Free Plasmid Midi Kit

Cat. #: M0018 Size: 10 rxn

Introduction

LiPure™ Endotoxin-Free Plasmid Midi Kit is specially designed to efficiently and rapidly extract plasmids from 15-50 ml bacterial cells. Based on conventional alkaline lysis method, the new and unique silicon membrane binds plasmid DNA efficiently and specifically, and each column can bind up to 250 ug plasmid DNA. Meanwhile by using a special buffer system and endotoxin-removal filters, impurities such as endotoxin, genomic DNA, RNA, proteins are effectively removed.

The plasmids obtained from the kit have high purity and high yield and are particularly suitable for transfection experiments. The plasmids can also be used for DNA sequencing, PCR, in vitro transcription, restriction endonuclease digestion and other experiments.

Package Information

Components	M0018
Buffer P1	30 ml
Buffer P2	30 ml
Buffer E3	30 ml
Buffer PS	15 ml
Buffer PW (concentrated)	10 ml
Endo-Free Buffer EB	30 ml
RNase A (10 mg/ml)	600 µl
Endo-Remover FX	50
Plungers	50
Spin Columns DX with Collection Tubes	10
Centrifuge Tubes (15 ml)	10

Not included in the kit: 100% ethanol; Isopropanol.

Storage

Store at Room temperature (15-30°C)

Preparation before the experiment and precautions

1. All components can be stored in a stable, dry, room temperature (15-30°C) environment for 1 year. The column can be stored at 2-8°C for longer storage time. Buffer P1 added with RNase A can be stored stably at 2-8°C for 6 months.

2. Add RNase A (all the RNase A provided in the kit) to Buffer P1 before use, mix well, and store at 2-8°C. Before use, it should be left at room temperature for a period and then used after returning to room temperature.

3. 100% ethanol should be added to the Buffer PW before the first use according to the instructions on the bottle label.

4. Before use, please check whether Buffer P2 and Buffer E3 are crystallized or precipitated. If there is any crystallization or precipitation, it can be dissolved in a 37°C water bath for several minutes.

5. Note that Buffer P2 and Buffer E3 contain irritating substances. Wear gloves during operation. Close the lid immediately after use.

6. The amount and purity of the extracted plasmid are related to factors such as bacterial culture concentration, strain type, plasmid size, and plasmid copy number.

7. The column that has been treated with Buffer PS is best used immediately, because long storage time after treatment will affect the column.

Protocol

1. Take 15-50 ml overnight bacteria culture and transfer it to a centrifuge tube (self-prepared). Collect the bacteria by centrifugation at 5,000× g for 10 minutes. Discard all the supernatant as much as possible.

2. Add 2.5 ml of Buffer P1 (please check if RNase A has been added) to the centrifuge tube with pellets. Mix well by pipetting or vortex to resuspend the pellet.

Note: If the bacteria pellet is not thoroughly resuspended, the lysis effect will be affected, and the amount and purity of extracted DNA will be lower.

3. Add 2.5 ml of Buffer P2 to the tube and invert gently for 8-10 times. Let the tube stand at room temperature for 3-5 minutes. At this point the solution should become clear and viscous.

Note: Mix gently and do not vortex violently to avoid interrupting the genomic DNA, resulting in the extracted plasmid mixed with genomic DNA fragments. If the solution does not become clear, it may indicate that the amount of bacteria may be too large and the lysis is not complete. The amount of bacteria should be reduced.

4. Add 2.5 ml of Buffer E3 to the tube and invert immediately for 8-10 times. A white flocculent precipitate should appear at this point.

Note: Mixed immediately after Buffer E3 is added to avoid local precipitation.

5. Put on the cap of the Endo-Remover FX. Pour the supernatant obtained from step 4 into Endo-Remover FX. After the white floc settles on the top of the solution, take off the cap, and push the filter (Plungers) slowly. Collect the flow-through in a clean 15 ml centrifuge tube (self-prepared) as much as possible.

6. Add 1/3 volume of isopropanol to the flow-through and mix by inverting.

7. Column equilibration: Add 1 ml of Buffer PS to the column (Spin Columns DX) with the collection tube, and centrifuge for 2 minutes at 2500× g. Discard the waste from the collection tube and return the column to the collection tube.

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8. Transfer the mixed solution in step 6 to an equilibrated column with a collection tube.

9. Centrifuge for 1 minute at 2500× g. Discard the waste from the collection tube and return the column to the collection tube.

Note: The maximum volume of the column is 4 ml, so it will take two times to transfer the solution obtained in step 8.

10. Add 2 ml Buffer PW to the column (please check if ethanol has been added first) and centrifuge at 2500× g for 1 minute. Discard the waste from the collection tube.

11. Repeat step 10.

12. Place the column back into the collection tube and centrifuge at 2500× g for 2 minutes. Discard the waste and leave the column to stand at room temperature for 5 minutes to thoroughly dry the column.

Note: The purpose of this step is to remove the residual ethanol in the column, and the residual of ethanol will affect the subsequent enzymatic reaction (enzyme digestion, PCR, etc.)

13. Place the column in a new 15 ml collection tube and add 0.5-1 ml Endo-Free Buffer EB to the middle of the membrane; Leave at room temperature for 2-5 minutes, then centrifuge at 2500× g for 2 minutes, and the plasmid is collected into a centrifuge tube. Store the plasmid at -20°C.

Note: 1) In order to increase the recovery efficiency of the plasmid, the elution can be added back to the column, leave at room temperature for 2-5 minutes, centrifuge at 2500× g for 2 minutes, and the plasmid is collected into a centrifuge tube.

2) When the plasmid is a low copy number plasmid or the size of the plasmid >10 kb, Endo-Free Buffer EB can be preheated in a water bath at 65-70°C to increase extraction efficiency.