LifeSct.com
Life Scientists' Service Center
Email: support@lifesct.com
Tel: 240-715-2985 Fax: 240-252-7376
14323 Woodcrest Dr., Rockville, MD 20853

LiPure™ Endotoxin-Free Plasmid Mini Kit

Cat. #: M0017 Size: 50 preps

Introduction

LiPure™ Endotoxin-Free Plasmid Mini Kit provides a simple, rapid and efficient method for the extraction of endotoxin-free plasmids. The contaminations such as endotoxin, genome DNA, RNA, and proteins can be maximumly removed. The operation is simple and convenient.

This kit is suitable for the extraction of 1-5 ml bacterial cells. Based on conventional alkaline lysis method, the new silicon membrane binds plasmid DNA efficiently and specifically. Each column can adsorb up to 40 μg of plasmid DNA. Special buffer systems and endotoxin removal columns can remove impurities such as endotoxins and proteins. The plasmids obtained from the kit are pure and stable and are particularly suitable for transfection experiments. The plasmids and can also be used for downstream experiments such as DNA sequencing, PCR, PCRbased mutations, in vitro transcription, transformation of bacteria, and restriction enzyme digestion.

Package Information

Components	M0017
Buffer P1	15 ml
Buffer P2	15 ml
Buffer E3	15 ml
Buffer PS	15 ml
Buffer PW (concentrated)	10 ml
Endo-Free Buffer EB	10 ml
RNase A (10 mg/ml)	150 μl
Endo-Remover FM with Collection Tubes	50
Spin Columns DM with Collection Tubes	50

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 stably in a dry environment at room temperature (15-30°C) 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 for 6 months at 2-8°C.

2. Before the first use, add whole RNase A solution to Buffer P1, mix, and store at 2–8°C. Leave buffer P1 at room temperature for a while before use, until it reaches room temperature.

3. 100% ethanol should be added to the Buffer PW before the first use, following the instructions on the reagent 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. Be careful not to touch the Buffer P2 and Buffer E3 directly. Close the cap 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.

Protocol

1. Take 1-5 ml of overnight bacteria culture and add it to a centrifuge tube (self-prepared). Centrifuge at 13,000 rpm (~16,200× g) for 30 seconds to collect the bacteria. Discard all the supernatant as much as possible.

2. Add 250 μl Buffer P1 (please check if RNase A was added) to the bacteria pellet. Mix well with a pipette or vortex to resuspend the pellet.

Note: If the pellet is not thoroughly resuspended, it will affect the lysis and the amount and purity of extracted plasmids will be lower.

3. Add 250 μl of Buffer P2 to the tube, then gently invert the tube upside and down for 8-10 times. Leave at room temperature for 3-5 minutes. At this point the solution should become clear and viscous.

Note: Mix gently and do not vortex, to avoid shearing 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 much and the lysis is not complete. The amount of bacteria should be reduced.

4. Add 250 μl Buffer E3 to the tube and invert immediately 8-10 times. At this point, white precipitation should be observed. Leave at room temperature for 5 minutes. After centrifuge at 13,000 rpm for 5 minutes, transfer the supernatant to a filter (Endo-Remover FM), centrifuge at 13,000 rpm for 1 minute, and the flow-through was collected in a centrifuge tube (selfprepared).

Note: Mix immediately after adding Buffer E3 to avoid local precipitation.

5. Add 225 μl of isopropanol to the filtrate and mix by inverting.

6. Column equilibration: Add 200 μl Buffer PS to the column (Spin Columns DM with collection tube). Centrifuge at 13,000 rpm for 1 minute. Discard the waste from the collection tube and put the column back to the collection tube.

7. Transfer the mixed solution of the filtrate and isopropanol in step 5 to an equilibrated column with collection tube.

LifeSct.com
Life Scientists' Service Center
Email: support@lifesct.com
Tel: 240-715-2985 Fax: 240-252-7376
14323 Woodcrest Dr., Rockville, MD 20853

8. Centrifuge at 13,000 rpm for 1 minute. Discard the waste from the collection tube and put the column back to the collection tube.

Note: the maximum volume of the column is 750 ul. If the sample volume is greater than 750 ul, add the sample multiple times.

9. Add 750 µl Buffer PW to the column (please check if 100% ethanol was added), centrifuge at 13,000 rpm for 1 minute, and discard the waste from the collection tube.

10. Put the column back to the collection tube and centrifuged at 13,000 rpm for 1 minute.

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.).

11. Place the column in a new collection tube and add 50-100 µl Endo-Free Buffer EB to the middle of the membrane; Leave at room temperature for 2-5 minutes, then centrifuge at 13,000 rpm 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 13,000 rpm 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.