
Endotoxin-free plasmid miniaturization kit

Project number: E665636

Storage conditions: room temperature.

Products content:

Component	E665636 50preps
BufferP1	15mL
BufferP2	15mL
BufferE3	15mL
BufferPS	15mL
BufferPW(concentrate)	10mL
Endo-FreeBufferEB	10mL
RNaseA (10 mg/mL)	150 μ L
Endo-RemoverFM	
withCollectionTubes	50
SpinColumnsDM	50
withCollectionTubes	

Product Description:

Endotoxin is a common contaminant in plasmid extraction, and since eukaryotic cells are very sensitive to endotoxin, the transfection efficiency of eukaryotic cells will be greatly reduced if the plasmid contains endotoxin. This kit provides a new method for simple, fast and efficient extraction of endotoxin-free plasmids, and the extracted plasmids maximize the removal of endotoxin, and can effectively remove genomic DNA, RNA, protein and other contaminants, and the operation is simple and convenient.

This kit is suitable for extracting 1-5mL of bacterial fluid, based on the lysis of cells by alkaline lysis method, the plasmid DNA is efficiently and specifically bound by the new silica-based plasmid membrane, each adsorption column can adsorb up to 40 μ g of plasmid DNA, and at the same time, special buffer system and endotoxin removal filtration columns are used to effectively remove endotoxins, proteins and other impurities. The plasmids obtained from this kit are of high purity and stable quality, and are particularly suitable for cell transfection, as well as downstream experiments such as DNA sequencing, PCR, PCR-based mutagenesis, in vitro transcription, transformation of bacteria, endonuclease digestion, and so on.

Self-contained reagents: anhydrous ethanol, isopropanol.

Lab prep and important notes:

1. All components can be stored stably in a dry, room temperature (15–30° C) environment for 1 year, the adsorption column can be stored at 2–8° C for a longer period of time, and BufferP1 with RNaseA can be stored stably at 2–8° C for 6 months.
2. Before the first use, add all the RNaseA solution into BufferP1, mix well, and store it at 2–8° C. Before use, leave it at room temperature for a period of time, and then use it after recovering to room temperature.
3. Anhydrous ethanol should be added to BufferPW according to the instructions on the label of the reagent bottle before first use.
4. Before use, please check whether BufferP2 and BufferE3 are crystallized or precipitated. If there is any crystallization or precipitation phenomenon, it can be clarified by water bath at 37°C for a few minutes.
5. Be careful not to touch BufferP2 and BufferE3 directly, and tighten the lid immediately after use.
6. The amount and purity of the extracted plasmid is related to the concentration of bacterial culture, strain type, plasmid size, plasmid copy number and other factors.

Operational Steps:

1. Take 1–5mL of the overnight culture, add it to a centrifuge tube (self-prepared), centrifuge at 13,000rpm (~16,200×g) for 30 seconds to collect the bacteria, and aspirate all the supernatant as much as possible.
2. Add 250 μL of BufferP1 to the centrifuge tube with the bacterial precipitate (please check that RNaseA has been added first) and mix well using a pipette or vortex shaker to suspend the bacterial precipitate.

Note: If the clumps are not thoroughly mixed, it will affect the lysis and make the extraction volume and purity low.

3. Add 250 μL of BufferP2 to the centrifuge tube, mix gently by turning up and down 8–10 times to fully lyse the organisms, and leave at room temperature for 3–5 minutes. At this point the solution should become clear and viscous.

Note: Mix gently, do not shake violently, so as not to interrupt the genomic DNA, resulting in genomic DNA fragments mixed in the extracted plasmid. If

If the solution does not become clear, it suggests that the amount of bacteria may be too large and the lysis is not complete, and the amount of bacteria should be reduced.

4. Add 250 μL of BufferE3 to the centrifuge tube, immediately mix upside down 8–10 times, when a white flocculent precipitate appears, and leave it at room temperature for 5 min. centrifuge at 13,000 rpm for 5 min. aspirate the supernatant, add the supernatant to a filtration column (Endo-RemoverFM), and then filter it

by centrifuging it at 13,000 rpm for 1 min, and the filtrate is collected in centrifuge tubes (supplied). The filtrate was collected in a centrifuge tube (self-contained).

Note: BufferE3 should be mixed immediately after addition to avoid localized precipitation.

5. Add 225 μ L of isopropanol to the filtrate and mix upside down.
6. Column equilibration: Add 200 μ L BufferPS to the adsorption column (SpinColumnsDM) that has been loaded into the collection tube, centrifuge at 13,000 rpm for 1 minute, pour off the waste liquid in the collection tube, and put the adsorption column back into the collection tube.
7. Transfer the mixture of filtrate and isopropanol from step 5 to the equilibrated adsorbent column (which has been loaded into the collection tube).
8. Centrifuge the column at 13,000 rpm for 1 minute, pour off the waste liquid from the collection tube and put the column back into the collection tube.

Note: The maximum volume of the adsorption column is 750 μ L, if the sample volume is larger than 750 μ L can be added in batches.

9. Add 750 μ L BufferPW to the adsorbent column (check that anhydrous ethanol has been added first), centrifuge at 13,000 rpm for 1 min, and pour off the waste liquid in the collection tube.
10. Place the adsorbent column back into the collection tube and centrifuge at 13,000 rpm for 1 minute.

Note: The purpose of this step is to remove residual ethanol from the adsorption column, which can interfere with subsequent enzymatic reactions (digestion, PCR, etc.).

11. Place the adsorbent column in a new collection tube, add 50–100 μ L Endo-FreeBufferEB to the middle part of the adsorbent membrane, leave it at room temperature for 2–5 minutes, centrifuge at 13,000 rpm for 2 minutes, and collect the plasmid solution into the centrifuge tube. -20° C to store the plasmid.

Attention:

To increase the recovery efficiency of the plasmid, the resulting solution can be reintroduced into the adsorbent column, left at room temperature for 2–5 minutes, centrifuged at 13,000 rpm for 2 minutes, and the plasmid solution collected into a centrifuge tube.

Endo-FreeBufferEB preheating in a $65-70^{\circ}$ C water bath when plasmid copy number is low or $>10\text{kb}$ can increase extraction efficiency.