
GoldHi EndoFree Plasmid Midi Kit

Project number: G665573

Storage conditions: room temperature (15–30° C)

Product content

Component	G665573–10T
Buffer P1	30ml
Buffer P2	30ml
Buffer E3	30ml
Buffer PS	15ml
Buffer PW (concentrate)	10ml
Endo-Free Buffer EB	30ml
RNase A (10mg/ml)	600μl
Endo-Remover FX	10
Plungers	10
Spin Columns DX with Collection Tubes	10
Centrifuge Tubes (15 ml)	10

Product Introduction

This kit is specially designed for the efficient and rapid extraction of plasmids from 15–50 ml of bacterial fluids. On the basis of cell lysis by alkaline lysis method, it adopts unique silicon matrix membrane adsorption technology to bind plasmid DNA efficiently and exclusively, and each adsorption column can adsorb up to 250 μg of plasmid DNA; at the same time, it adopts a special buffer system and endotoxin removal filter to effectively remove endotoxin, genomic DNA, RNA, protein and other impurities. The plasmids obtained from this kit are of high purity and stable quality, and can be used for cell transfection, as well as DNA sequencing, PCR, in vitro transcription, endonuclease digestion and other experiments.

Self-contained reagents: anhydrous ethanol, isopropanol.

Pre-experiment Preparation and Important Notes

1. All components are stable for 1 year in a dry, room temperature (15–30° C) environment, and longer by placing the adsorption columns at 2–8° C. Buffer P1 with RNase A is stable for 6 months at 2–8° C.
2. Before the first use, add all of the RNase A solution to Buffer P1, mix well, and store at 2–8° C. Before use, it needs to be left at room temperature for a period of time, return to room temperature and then use.
3. Anhydrous ethanol should be added to Buffer PW before the first use according to the instructions on the reagent bottle label.
4. Please check Buffer P2 and Buffer E3 for crystallization or precipitation before use. If there is any crystallization or precipitation, the clarification can be restored by taking a water bath at 37°C for a few minutes.
5. Be careful not to touch Buffer P2 and Buffer E3 directly, and tighten the lid immediately after use.
6. The amount and purity of extracted plasmid is related to the concentration of bacterial culture, strain type, plasmid size, plasmid copy number and other

factors.

7. The adsorption columns treated with Buffer PS should be used immediately to avoid leaving them for too long.

Operation steps

1. Take 15–50 ml of fresh bacterial solution from the overnight culture, add it to a centrifuge tube (self-prepared) and centrifuge at $5000 \times g$ for 10 minutes to collect the bacteria, and aspirate all the supernatant as much as possible.

2. Add 2.5 ml of Buffer P1 to the centrifuge tube in which the bacterial precipitate has been left (please check that RNase A has been added first) and suspend the bacterial precipitate by mixing thoroughly using a pipette or vortex shaker. Note: If the bacterial mass is not thoroughly mixed, it will affect the lysis effect and make the extraction amount and purity low.

3. Add 2.5 ml of Buffer P2 to the centrifuge tube, mix gently 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 vigorously, so as not to interrupt the genomic DNA and cause genomic DNA fragments to be mixed in the extracted plasmid. 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 2.5 ml of Buffer E3 to the centrifuge tube and mix immediately by turning up and down 8–10 times, at which time a white flocculent precipitate appears. Note: Buffer E3 should be mixed immediately after addition to avoid localized precipitation.

5. Install the cap of the filter (Endo-Remover FX), transfer the solution obtained in step 4 to the filter, wait until the white flocculent precipitate floats on the upper layer of the solution, remove the cap of the filter, align the filter with a clean 15 ml centrifuge tube (supplied), and slowly push the handle (Plungers) to filter, so that as much as possible of the solution passes through, and the filtrate is collected in the centrifuge tube.

6. Add 1/3 solution volume of isopropanol to the filtrate and mix upside down.

7. Column Equilibrium: Add 1 ml Buffer PS to the adsorption column (Spin Columns DX) that has been loaded into a 15 ml centrifuge tube, centrifuge for 2 minutes at $2500 \times g$. Pour off the waste liquid from the centrifuge tube and put the adsorption column back into the centrifuge tube.

8. The mixture of filtrate and isopropanol from step 6 was transferred to the equilibrated adsorption column (which had been loaded into a collection tube).

9. Centrifuge at $2500 \times g$ for 1 minute, pour off the waste solution in the collection tube and put the adsorption column back into the collection tube. Note: The maximum volume of the adsorption column is 4 ml, so the solution obtained in step 8 is passed through the column in 2 times.

10. Add 2 ml of Buffer PW to the adsorption column (please check that anhydrous ethanol has been added first), centrifuge at $2500 \times g$ for 1 min, and pour off the waste liquid in the collection tube.

11. Repeat step 10.

12. The adsorbent column was put back into the collection tube and centrifuged at $2500 \times g$ for 2 min, the waste liquid was poured off, and the column was

left to dry at room temperature for 5 min.

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.)

13. Place the adsorption column in a new 15 ml centrifuge tube, add 0.5–1 ml Endo-Free Buffer EB to the middle of the adsorbent membrane, leave it at room temperature for 2–5 minutes, centrifuge it at $2500 \times g$ for 2 minutes, and collect the plasmid solution into the centrifuge tube. -20°C to store the plasmid.

Note: 1) In order to increase the recovery efficiency of the plasmid, the obtained solution can be reintroduced into the adsorption column, left at room temperature for 2–5 minutes, centrifuged at $2500 \times g$ for 2 minutes, and the plasmid solution can be collected into a centrifuge tube.

2) When the plasmid copy number is low or $>10\text{kb}$, Endo-Free Buffer EB can increase the extraction efficiency by preheating at $65\text{--}70^{\circ} \text{C}$ in a water bath.