Gold Ultra Endotoxin Free Plasmid Macro Extraction Kit

(Negative Pressure Method)

Project number: G665655

Storage conditions: room temperature.

Products content:

Component	G665655 2preps	G665655 10preps
BufferP1	30ml	125ml
BufferP2	30ml	125ml
BufferE3	30ml	125ml
BufferPS	15ml	30ml
BufferPW (concentrate)	10ml	50ml
Endo-FreeBufferEB	10ml	30ml
RNaseA (10 mg/ml)	0.6ml	2ml
Plungers	2	10
Endo-RemoverFQ	2	10
DNA-BindingTubes	2	10
VacConnectors	2	10
CentrifugeTubes (50 ml)	2	20

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 rapid and easy large-scale plasmid preparation. It can process 100-300 ml of bacterial broth at a time and obtain up to 2 mg of transfection-grade plasmid DNA, and it can purify multiple samples at the same time using a vacuum device, and plasmid extraction can be completed in 45 minutes, which effectively reduces the time for manual operation. Special buffer system and endotoxin removal filter can effectively remove endotoxin, genomic DNA, RNA, protein and other impurities. With high purity and large extraction volume, this kit is especially suitable for cell transfection, and can also be used for DNA sequencing, PCR, in vitro transcription, endonuclease digestion and other experiments.

Self-contained reagents: anhydrous ethanol, isopropanol, vacuum pump, waste liquid collection device, vacuum purification device.

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. Add RNaseA to BufferP1 before use (add all the RNaseA provided in the kit), mix well.

3. Store at 2-8° C. Allow to stand at room temperature for a period of time before use, return to room temperature and then use. 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℃ for a few minutes.

5. Note that BufferP2 and BufferE3 contain irritating substances, please wear gloves to operate, and the lid should be tightened immediately after use. 6. It is best to use the adsorption columns treated with BufferPS immediately to avoid placing them for a long time and affecting the effect of using them.

7. Please prepare waste liquid collection device/buffer bottle CWE100, vacuum purification device CWE200, vacuum pump CWE300.

Vacuum pump waste liquid collection device / buffer bottle vacuum purification device

Operational Steps:

1. Take 100-300 ml of the overnight culture, add it to a centrifuge tube (self-prepared), centrifuge at 12,000 × g for 2-3 minutes to collect the bacteria, and try to aspirate all the supernatant.

2. Add 12 ml 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 bacterial mass is not thoroughly mixed, it will affect the lysis effect and make the extraction volume and purity low.

3. Add 12 ml 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 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 12 ml of BufferE3 to the centrifuge tube and immediately mix upside down 8-10 times, when a white flocculent precipitate appears, and leave for 5 minutes at room temperature. Pour all the solution into an endotoxin removal filter (Endo-RemoverFQ), slowly push the handle (Plungers) to filter, and collect the filtrate in clean 50 ml centrifuge tubes (CentrifugeTubes).

Note: 1) BufferE3 should be mixed immediately after addition to avoid localized precipitation.

2) If excessive precipitation occurs after addition of E3, centrifuge at 12,000 x g for 10 minutes and then pour the supernatant solution into an endotoxin removal filter.

5. Add 0.3 times the volume of filtrate to the filtrate with isopropyl alcohol and mix upside down.

Note: Adding too much isopropanol can easily lead to RNA contamination.

6. Connect the negative pressure device correctly by connecting the connecting tubes (VacConnectors) to the DNA-BindingTubes and inserting them into the sockets of the negative pressure device.

CAUTION: Ensure that the connecting tube and adsorption column are securely connected to prevent air leakage.

7. Column equilibrium: Add 2 ml BufferPS to the DNA adsorption column (DNA-BindingTubes), turn on and adjust the negative pressure to -300~-700 mbar, and aspirate the solution on the column.

8. Transfer the mixture of filtrate and isopropanol from step 5 to an equilibrated adsorption column and aspirate the solution from the column.

9. Add 10 ml of BufferPW to the DNA adsorption columns (DNA-BindingTubes) (please check that anhydrous ethanol has been added first) and aspirate the solution from the column.

10. Repeat step 9.

11. Keep the negative pressure suction for 10 minutes, remove the residual rinsing liquid in the adsorption membrane, dry the adsorption membrane. If more than 6 samples at a time, the negative pressure suction time can be extended appropriately. After the adsorption membrane is completely dry, turn off the negative pressure switch.

Attention:

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

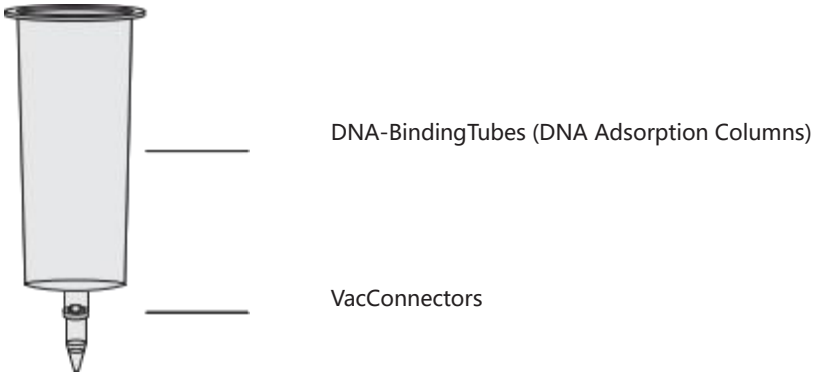
(2) According to the adsorption membrane drying, decide the negative pressure device suction time. If not thoroughly dried, can also increase the drying step, 65 ℃ drying 30min to thoroughly dry the adsorption membrane residual solution.

12. When the pressure is restored to 0 mbar, remove the adsorbent column, place the DNA adsorbent column in a new 50 ml centrifuge tube (CentrifugeTubes), add 1-3 ml of Endo-FreeBufferEB to the middle of the adsorbent membrane, leave it at room temperature for 2-5 min, centrifuge it for 5 min at 12,000×, and collect the plasmid solution into the centrifuge tube. . -20° C to preserve the plasmid.

Attention:

1) In order to increase the recovery efficiency of the plasmid, the obtained solution can be reintroduced into DNA adsorption columns (DNA-BindingTubes), left at room temperature for 2-5 minutes, centrifuged at 12,000 x g for 5 minutes, and the plasmid solution can be collected into centrifuge tubes.

2) When the plasmid copy number is low or >10kb, Endo-FreeBufferEB can increase the extraction efficiency by preheating at 65-70° C in a water bath.



Schematic diagram of the connection between DNA adsorption column and connecting tube