Gold Ultra Endotoxin Free Plasmid Macro Extraction Kit

Project number: G665587

Storage conditions: room temperature.

Products content

Component	G665587 2preps	G665587 10preps
BufferP1	30mL	125mL
BufferP2	30mL	125mL
BufferE3	30mL	125mL
BufferPS	15mL	30mL
BufferPW(concentrate)	10mL	50mL
Endo-FreeBufferPW	30mL	125mL
Endo-FreeBufferEB	10mL	30mL
RNaseA (10 mg/mL)	600μL	2mL
SpinColumnsDZwithCollectionTubes	2	10
CentrifugeTubes (50mL)	2	10

Product Description:

Endotoxin is a common contaminant in plasmid extraction. 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 and efficient extraction of endotoxin-free plasmids. Based on the lysis of cells by alkaline lysis method, it adopts a unique silica-based plasmid membrane adsorption technology to efficiently and exclusively bind plasmid DNA; at the same time, it adopts a special buffer system and a de-endotoxin buffer to efficiently remove endotoxin, genomic DNA, RNA, proteins, and other impurities. It can process 100-300mL of bacterial fluid each time, and obtain up to 2mg of transfection-grade plasmid DNA, and the whole extraction process only takes 50 minutes. 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.

Precautions:

1. All components can be stably stored in a dry, room temperature (15-30° C)

environment for 1 year, and the adsorption column can be stored for a longer period of time by placing it at 2-8° C. BufferP1 with RNaseA added can be stably stored at 2-8° C for 6 months. 2.BufferP1 should be added with RNaseA before use (add all of the RNaseA supplied in the kit), mix well, and store at 2 BufferP1 should be stored at 2-8° C. Before use, it should be left at room temperature for a period of time and used after returning 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, the clarification can be restored by taking a water bath at 37℃ for a few minutes.

5. Note that BufferP2 and BufferE3 contain irritating substances, please wear gloves to operate, and tighten the lid immediately after use.

6. It is better to use BufferPS treated adsorption columns and leave them for 15-30 minutes before passing the mixture through the column, it is not recommended to leave them for more than 30 minutes for use.

7. The amount and purity of the extracted plasmid is related to the bacterial culture concentration, strain type, plasmid size, plasmid copy number and other factors.

8. The amount of bacterial liquid for plasmid extraction should not exceed the recommended volume in Table 1 and Table 2, and the dosage of P1, P2 and E3 solution for high copy plasmid extraction is 12mL; while for low copy plasmid extraction, the dosage of P1, P2 and E3 solution should be adjusted according to the amount of bacterial liquid for the dosage of P1, P2 and E3 solution, which can be referred to in Table 3:

Table 1

Maximum amount of bacterial solution to be used for high copy plasmids						
wet weight	ODV	OD600=2	OD600=4	OD600=6	OD600=8	OD600=10
1.65g	1000	500mL	250mL	166mL	125mL	100mL

Table 2

Maximum amount of bacterial solution to be used for low-copy plasmids						
wet weight	ODV	OD600=2	OD600=4	OD600=6	OD600=8	OD600=10
2.0g	1200	600mL	300mL	200mL	150mL	120mL

Note: ODV = OD600 × V, V is the volume of bacterial liquid (mL); recommended OD600 is 1-4.

9. The volume of lysate is recommended for low-copy plasmids, see the table below for details:

Table 3

wet weight	2.0g	1.6g	1.3g	1.0g
volume of bacterial liquid	300mL	250mL	200mL	150mL
P1, P2, E3 usage	24mL	20mL	16mL	12mL

Note: Use OD600=4 as an example.

Operational Steps:

1. Take 150mL of the overnight culture, add it to a centrifuge tube (self-prepared), centrifuge at 12,000×g for 2-3 minutes to collect bacteria, and try to aspirate all the supernatant.

2. Add 12 mL of BufferP1 to the centrifuge tube with the bacterial precipitate (check to see if RNase A has been added) 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 amount and purity low. For low-copy plasmids, the action of bacterial liquid

The amount of P1, P2 and E3 used needs to be increased proportionally for larger quantities, please refer to Table 3.

3. Add 12 mL of Buffer P2 to the centrifuge tube and mix gently up and down 8-10 times to fully lyse the organisms and allow to stand at room temperature for 5 minutes. At this point the solution should become clear and viscous.

Note: Mix gently, do not shake vigorously to avoid interrupting the genomic DNA and causing 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 incomplete, and the amount of bacteria should be reduced.

4. Equilibrate the column at this point: add 2 mLBuffer PS to the adsorbent column (Spin Columns DZ) that has been loaded into the collection tube, centrifuge at 12,000 × g for 2 minutes, pour off the waste liquid from the collection tube, and place the adsorbent column back into the collection tube. Note: After step 4 column equilibration is complete (Buffer PS over column) until used in step 7 (supernatant and isopropanol mixture over column) it is recommended to have the

A time lag of 15-30 minutes can lead to higher extraction rates.

5. Add 12 mL of Buffer E3 to the centrifuge tube and immediately mix 8-10 times, a white flocculent precipitate appears, let stand at room temperature for 5 min. centrifuge at 12,000 x g for 10 min. transfer the supernatant to a clean centrifuge tube (supplied) taking care not to bring in the precipitate.

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

6. Add 0.3 times the supernatant volume of isopropanol and mix upside down.

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

7. Transfer the supernatant mixed solution with isopropanol to the adsorbent column DZ (which has been loaded into the collection tube) that has been equilibrated in

step 4. Centrifuge the column at 12,000 x g for 2 minutes, pour off the waste solution from the collection tube, and place the adsorbent column back into the collection tube.

Note: The maximum volume of the adsorption column is 15 mL, so the solution obtained in step 7 is passed through the column several times. If the centrifuge rotor inclination is large, it is recommended that the volume of solution added to the adsorption column does not exceed 10 mL to prevent leakage.

8. Add 10mLBufferPW to the adsorbent column (please check that anhydrous ethanol has been added first), centrifuge at 12,000 x g for 2 minutes, pour off the waste liquid in the collection tube, and put the adsorbent column back into the collection tube.

9. Repeat step 8.

10. Add 10 mLEndo-FreeBufferPW to the adsorbent column and centrifuge at 12,000 × g for 2 min. Pour off the waste liquid in the collection tube and put the adsorbent column back into the collection tube.

11. Place the adsorbent column back into the collection tube and centrifuge at 12,000 x g for 5 minutes, pour off the waste liquid, and leave the column at room temperature for a few minutes to thoroughly dry out any rinse solution remaining in the adsorbent column.

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.).

12. Place the adsorbent column in a new centrifuge tube, add 1-3 mLEndo-FreeBufferEB to the middle part of the adsorbent membrane, leave it at room temperature for 2-5 minutes, and centrifuge at 12,000 × g for 5 minutes to 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 12,000 × g for 5 minutes, and the plasmid solution collected into a centrifuge tube.

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.