Gold Ultra Plasmid Mini-Lift Kit

Project number: G665666

Storage conditions: -20° C.

Product Content:

Component	G665666 200preps
BufferP1	60ml
BufferP2	60ml
BufferE3	60ml
BufferPW(concentrate)	25ml
BufferEB	30ml
RNaseA (10mg/ml)	600 μl
SpinColumns DM	200
withCollectionTubes	200

Product Description:

This kit is suitable for extracting 1-5 ml of bacterial fluid, on the basis of lysis of cells by alkaline lysis method, it can bind plasmid DNA efficiently and specifically by the new silica-based plasmid membrane, and each adsorption column

can adsorb up to 40 μg of plasmid DNA, and at the same time, it adopts a special buffer system to remove the protein and other impurities effectively. The yield and purity of plasmids obtained from this kit are high and the quality is stable, which is suitable for downstream experiments such as cell transfection, 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 stably stored in dry, room temperature (15-30℃) environment for 1 year, the adsorption column can be stored at 2-8℃ for a longer period of time, and BufferP1 with RNaseA can be stably stored at 2-8℃ for 6 months.
2. BufferP1 add RNaseA before use (add all the RNaseA provided in the kit), mixing Mix well and store at 2-8° C. Allow to stand at room temperature for a period of time before use, return to room temperature and use.3. Anhydrous ethanol should be added to BufferPW according to the instructions on the label of the reagent bottle before first use.
4. Please check BufferP2 and BufferE3 for crystallization or precipitation before use. If there is any crystallization or precipitation phenomenon, it can be clarified by water bath at 37℃ for a few minutes to restore the clarification.
5. Note that BufferP2 and BufferE3 contain irritating substances, please wear gloves to operate, 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 maximum volume of SpinColumnsDM is 750 μl, if the sample volume is larger than 750 μl can be added in batches.

Operational Steps:

1. Take 1-5 ml of the overnight culture, add it to a centrifuge tube (self-prepared), and centrifuge at 13,000 rpm (~16,200×g) for 1 minute to collect bacteria, and aspirate all the supernatant as much as possible.
2. Add 200 μl BufferP1 to the centrifuge tube with the bacterial precipitate (please check if 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 200 μl BufferP2 to the centrifuge tube and mix gently up and down 8-10 times to fully lyse the organisms. At this point the solution should become clear and

sticky.

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, the amount of bacteria should be reduced or the amount of P1, P2, E3 and isopropanol should be increased proportionally.

4. Add 200 μl of BufferE3 to the centrifuge tube and immediately mix upside down 8-10 times, when a white flocculent precipitate appears. centrifuge at 13,000 rpm for 5 minutes.

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

5. After adding 260 μl of isopropanol to the adsorption column (SpinColumnsDM) that has been loaded into the collection tube, immediately add the supernatant collected in step 4 and mix upside down.

Note: The supernatant should be added and mixed immediately after the addition of isopropanol to avoid isopropanol dripping into the collection tube after a long period of time. The maximum volume of the adsorption column is 750 μl. If the sample volume is larger than 750 μl, the isopropanol and supernatant can be collected in a centrifuge tube (supplied) and mixed well, and passed through the column in batches.

6. 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. Add 400 μl BufferPW to the adsorption column (please check that anhydrous ethanol has been added first), centrifuge at 13,000 rpm for 1 minute, and pour off the waste liquid in the collection tube.

8. Place the adsorbent column in a new collection tube, add 50-100 μl of BufferEB to the middle of the adsorbent membrane, and centrifuge at 13,000 rpm for 1 minute to collect the plasmid solution into the centrifuge tube. Store the plasmid at -20° C.

Attention:

1) In order to increase the recovery efficiency of the plasmid, the obtained 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 can be collected into a centrifuge tube.

2) When the plasmid copy number is low or >10kb, BufferEB is preheated in a 65-70° C water bath, which can increase the extraction efficiency.