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GoldHi EndoFree Plasmid Maxiprep Kit

Catalog Number: CW2104S (2 preps)
CW2104M (10 preps)

Storage Condition: Room temperature (15-30°C)

Kit Components:

Component	CW2104S (2 preps)	CW2104M (10 preps)
Buffer P1	30 ml	125 ml
Buffer P2	30 ml	125 ml
Buffer E3	30 ml	125 ml
Buffer PS	15 ml	30 ml
Buffer PW (concentrated)	10 ml	50 ml
Endo-Free Buffer EB	10 ml	30 ml
RNase A (10 mg/ml)	600 ul	2 ml
Plungers	2	10
Endo-Remover FQ	2	10
Spin Columns DQ with Collection Tubes	2	10
Centrifuge Tubes (50 ml)	2	10

Product Introduction:

Endotoxin is a common contaminant in plasmid extraction. Because eukaryotic cells are very sensitive to endotoxin, the transfection efficiency will be greatly reduced if endotoxin is contained in the plasmid. This kit provides a simple, rapid and efficient method for the extraction of endotoxin-free plasmids. Based on conventional alkaline lysis method, the new and unique silicon membrane binds plasmid DNA efficiently and specifically. At the same time by using a special buffer system and endotoxin-removal filters, impurities such as endotoxin, genomic DNA, RNA, proteins are effectively removed.

100-300 ml of bacterial culture can be processed each time, and up to 2 mg of transfection-grade plasmid DNA can be obtained. The entire extraction process takes only 50 minutes. The plasmids obtained from the kit have high purity and high yield and are particularly suitable for transfection experiments. The plasmids can also be used for DNA sequencing, PCR, in vitro transcription, restriction endonuclease digestion and other experiments.

Not included in the kit: 100% ethanol; Isopropanol.

Preparation before the experiment and important notes:

1. All components can be stored in a stable, dry, room temperature (15-30°C) environment for 1 year. The column can be stored at 2-8°C for longer storage time. Buffer P1 added with RNase A can be stored stably at 2-8°C for 6 months.
2. Add RNase A (all the RNase A provided in the kit) to Buffer P1 before use, mix well, and store at 2–8 °C. Before use, it should be left at room temperature for a period of time and then used after returning to room temperature.
3. 100% ethanol should be added to the Buffer PW before the first use according to the instructions on the bottle label.
4. Before use, please check whether Buffer P2 and Buffer E3 are crystallized or precipitated. If there is any crystallization or precipitation, it can be dissolved in a 37°C water bath for several minutes.
5. Note that Buffer P2 and Buffer E3 contain irritating substances. Wear gloves during operation. Close the lid immediately after use.
6. The column that has been treated with Buffer PS is best used immediately, because long storage time after treatment will affect the column.

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

Protocol:

1. Take **150 ml** overnight bacteria culture and transfer it to a centrifuge tube (self-prepared). Collect the bacteria by centrifugation at **12,000 ×g** for **2-3** minutes. Discard all the supernatant as much as possible.
2. Add **12 ml of Buffer P1** (please check if RNase A has been added) to the centrifuge tube with pellets. Mix well by pipetting or vortex to resuspend the pellet.
   Note: If the bacteria pellet is not thoroughly resuspended, the lysis effect will be affected, and the amount and purity of extracted DNA will be lower.
3. Add **12 ml of Buffer P2** to the tube and invert gently for **8-10** times. Let the tube stand at room temperature for **3-5** minutes. At this point the solution should become clear and viscous.
   Note: Mix gently and do not vortex violently to avoid interrupting the genomic DNA, resulting in the extracted plasmid mixed with genomic DNA fragments. If the solution does not become clear, it may indicate that the

amount of bacteria may be too large and the lysis is not complete. The amount of bacteria should be reduced.

4. Add **12 ml of Buffer E3** to the tube and invert immediately for **8-10** times. A white flocculent precipitate should appear at this point. Allowed to stand at room temperature for **5** minutes. Centrifuge at **12,000 ×g** for **10** minutes. Pour the supernatant into **Endo-Remover FQ**, slowly push the filter (**Plungers**), and collect the flow-through in a clean **50 ml** centrifuge tube (self-prepared).

   Note: Mixed immediately after Buffer E3 is added to avoid local precipitation.
5. Add **0.3 times volume of isopropanol** to the flow-through and mix by inverting.

   Note: Adding too much isopropanol can easily lead to RNA contamination.
6. Column equilibration: Add **2 ml of Buffer PS** to the column (**Spin Columns DQ**) with the collection tube, and centrifuge for **2** minutes at **12,000 ×g**. Discard the waste from the collection tube and return the column to the collection tube.
7. Transfer the mixed solution in **step 5** to an equilibrated column with a collection tube.

8. Centrifuge for **2** minutes at **6,000-12,000 ×g**. Discard the waste from the collection tube and return the column to the collection tube.

   Note: The maximum volume of the column is 15 ml, so it will take multiple times to transfer the solution obtained in step 7. It is recommended that the volume of the solution added to the column each time does not exceed 10 ml to prevent leakage.

9. Add **10 ml Buffer PW** to the column (please check if ethanol has been added first) and centrifuge at **6,000-12,000 ×g** for **2** minutes. Discard the waste from the collection tube.

10. Repeat step **9**.

11. Place the column back into the collection tube and centrifuge at **12,000 ×g** for **5** minutes. Discard the waste and leave the column to stand at room temperature for a few minutes to thoroughly dry the column.

    Note: The purpose of this step is to remove the residual ethanol in the column, and the residual of ethanol will affect the subsequent enzymatic reaction (enzyme digestion, PCR, etc.)

12. Place the column in a new collection tube and add **1-3 ml Endo-Free Buffer EB** to the middle of the membrane; Leave at room temperature for **2-5** minutes, then centrifuge at **12,000 ×g**

for **5** minutes, and the plasmid is collected into a centrifuge tube. Store the plasmid at -20°C.

Note: 1) In order to increase the recovery efficiency of the plasmid, the elution can be added back to the column, leave at room temperature for 2-5 minutes, centrifuge at 12,000 ×g for 5 minutes, and the plasmid is collected into a centrifuge tube.

2) When the plasmid is a low copy number plasmid or the size of the plasmid >10 kb, Endo-Free Buffer EB can be preheated in a water bath at 65-70°C to increase extraction efficiency.