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GoldVac EndoFree Plasmid Maxiprep Kit (Negative Pressure Method)

Catalog Number: CW2107S (2 preps); CW2107M (10 preps)

Storage Condition: Room temperature (15-30℃)

Kit Components:

Component	CW2107S	CW2107M
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.

100-300 ml of bacterial culture can be processed each time, and up to 2 mg of transfection-grade plasmid DNA can be obtained. Multiple samples can be handled simultaneously by use of a vacuum device and the whole process takes only 45 minutes, which effectively reduces hand-on time.

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.

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; Vacuum and Pump apparatus.

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’s performance.

7. Please prepare the vacuum pump (CWW0001), waste collection apparatus (CWW0003), and vacuum manifold (CWW0002). It is recommended to use the CoWin Biosciences' products.

Vacuum Pump Waste Collection Vacuum Manifold

Protocol:

1. Take **100-300 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. Leave the tube to 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. Pour the supernatant into **Endo-Remover FQ**, slowly push the **Plungers**, and collect the flow-through in a clean **50** ml centrifuge tube (self-prepared).

   Note: 1) Mixed immediately after Buffer E3 is added to avoid local precipitation. 2) After adding Buffer E3, if there is excessive precipitation, it can be centrifuged at 12,000×g for 10 minutes, and then pour the supernatant solution into the Endo-Remover FQ.
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. Connect the negative pressure device properly; Connect the connector to the column and insert them into the socket of the vacuum device.

   Note: Make sure that the connector and column are firmly connected to prevent air leakage.

7. Column equilibration: Add **2 ml of Buffer PS** to the column and turn on the vacuum apparatus to adjust the negative pressure to **-300 to -700 mbar**.
8. Transfer the mixed solution in step **5** to an equilibrated column.
9. Add **10 ml Buffer PW** to the column (please check if ethanol has been added first).
10. Repeat step **9**.
11. Keep the negative pressure for **10** minutes to dry the membrane of the column. If the membrane is still wet, keep the vacuum for **5** more minutes to dry the membrane completely. Turn off the vacuum when the membrane is dry.

    Note: 1) 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.)

2) The usage time of vacuum is decided by the condition of the membrane. If the membrane is still wet, the column can be put into 65°C incubator for 30 minutes to dry it completely.

12. When the pressure returns to **0 mbar**, take off the column and place it in a new **50 ml** collection tube. 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 the collection 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.

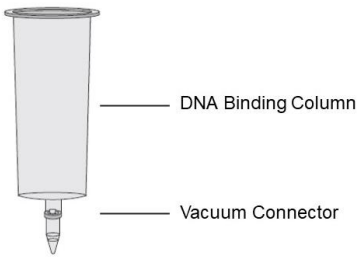

Diagram of connection between the column and connector