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PurePlasmid Miniprep Kit

Catalog Number: CW0500S (50 preps); CW0500M (200 preps)

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

Kit Components:

Component	CW0500S (50 preps)	CW0500M (200 preps)
Buffer P1	15 ml	60 ml
Buffer P2	15 ml	60 ml
Buffer N3	20 ml	80 ml
Buffer PB	10 ml	35 ml
Buffer PW (concentrated)	6 ml	25 ml
Buffer EB	10 ml	30 ml
RNase A (10 mg/ml)	150 ul	600 ul
Spin Column DM with Collection tube	50	200

Product Introduction:

The kit is suitable for the extraction of 1-5 ml bacterial cells. Based on the traditional alkaline lysis method, the kit uses a unique silica matrix membrane adsorption technology and reagent formulation. Each column can adsorb up to 30 ug of plasmid DNA, through the effective and specific binding of plasmid DNA under high salt condition. Proteins, genomes, RNA and other impurities can be maximumly removed. The plasmid DNA extracted can be directly used for biological experiments such as cell transfection, PCR, enzyme digestion, sequencing, and ligation.

Not included in the kit: 100% ethanol.

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 a longer time; Buffer P1 added with RNase A can be stored at 2-8°C for 6 months.
2. Before the first use, add all RNase A solution to Buffer P1, mix well and store at 2–8°C. Before use, leave it at room temperature for a period of time and then use after it returns 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. If Precipitations are found in Buffer P2, Buffer N3 and Buffer before use, put them in a 37°C water bath for a few minutes, until the precipitations dissolve (do not shake Buffer P2 vigorously).
5. Be careful not to touch the Buffer P2, Buffer N3 and Buffer PB directly, and close the cap immediately after use.
6. The yield and purity of the extracted plasmid are related with the bacterial culture concentration, strain type, plasmid size, plasmid copy number and other factors.

Protocol:

1. Transfer **1-5 ml** overnight bacteria culture to a centrifuge tube (self-prepared). Centrifuge at **13,000 rpm** (~**16,200 ×g**) for **30** seconds to collect the bacterial pellet. Discard the supernatant as much as possible.
2. Add **250 µl Buffer P1** (please check if RNase A was added first) to the tube with pellets. Mix thoroughly with a pipette or vortex.

   Note: If the bacteria pellet is not completely resuspended, it will affect the lysis effect, resulting in low yield and purity.

3. Add **250 µl Buffer P2** to the tube, gently invert the tube **4-6** times and mix well. The solution should become clear and viscous now.
   Note: Mix gently and do not vortex violently to avoid interrupting the genomic DNA, resulting in the extracted plasmid mixed with genomic DNA fragments. The time for this step should not exceed 5 minutes to avoid damage to the plasmid.
4. Add **350 µl of Buffer N3** to the tube and invert gently **8-10** times to mix well. A white flocculent precipitate should appear now. Centrifuge at **13,000 rpm** for **5** minutes.
   Note: It should be mixed immediately after Buffer N3 addition, to avoid local precipitation.
5. Transfer the supernatant obtained in step **4** to a **spin column DM** with a collection tube. Centrifuge at **13,000 rpm** for **30** seconds. Discard the waste from the collection tube and put the column back to the collection tube.
6. Add **150 µl Buffer PB** to the column and centrifuge at **13,000 rpm** for **30** seconds.
7. Add **400 µl Buffer PW** (please check if 100% ethanol was added) to the column; Centrifuge at **13,000 rpm** for **1** minute and discard the waste from the collection tube.

8. Place the column in a new collection tube and add **50-100 µl Buffer EB** to the middle of the membrane; Leave at room temperature for **2** minutes, then centrifuge at **13,000 rpm** for **1** minute, and the plasmid is collected into the 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 13,000 rpm for 2 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, Buffer EB can be preheated in a water bath at 65-70°C to increase extraction efficiency.