Note: *for laboratory research use only*

M13 Phage DNA Rapid Extraction Kit (Spin-column)

A kit for the isolation of single strand DNA from M13 phase

Cat. #: DP2301 (50 preps)

Signalway

Signalway Biotechnology

Ⅰ.Kit Content, Storage and Stability

Content	Storage	50 preps (DP2301)
Buffer MB	RT	25 ml
Buffer WB	RT	15 ml
		Add 60ml ethanol before use
Buffer EB	RT	10 ml
Spin-column AC	RT	50
Collection Tube (2ml)	RT	50

All reagents are stable for 18 months at RT if stored properly.

Reminder:

1 · ***Add ethanol to Buffer WB before use, mix adequately, and then check the box on the label showing ethanol has been added!***

2 · Buffer MB may precipitate under low temperature. Incubate to 37°C for a few minutes **until buffer is clear**, and then let the buffer cool to RT before use.

3 · Keep the reagent lids tightly caped when not in use to prevent evaporation, oxidation, and changes in pH.

Ⅱ.Principle:

The infected bacterial culture is centrifuged to pellet the bacterial cells, and high-salt buffer is added to the supernatant. Phage DNA is selectively adsorbed to silica membrane in a spin-column. Contaminants such as proteins and polysaccharides are efficiently removed by two wash steps. Pure phage DNA is eluted in buffer EB or DNase-free water.

Ⅲ.Features:

1・Rapid and convenient. Isolation can be finished in 10 minutes.

2・The kit does not contain poisonous phenol. No need for ethanol precipitation. Multi-elution can ensure highly-purified DNA, which can be applied to all kinds of molecular biology experiments, such as PCR, Southern-blot, restriction enzyme digestion, and mammalian cell transfections.

3・Excellent yield (up to 3μg/800μl culture).

Ⅳ.Notes

Please read this section before your experiment.

1. All the centrifugation can be performed at room temperature.
2. Buffer MB contains a corrosive compound; please wear latex gloves to avoid contact with skin, eyes, and cloth**. If contact occurs, wash with water or physiological saline.**
3. Purified DNA typically has A260/A280 ratio between 1.7 and 1.9, which is suitable for sequencing and other biological applications.
4. Set a water bath to 60-70℃ before use.

Ⅴ.Procedure

Before Starting

- ***Dilute Buffer WB with 60 ml absolute ethanol, vortex adequately, then the check the box on the bottle to indicate the ethanol has been added!***

1. Collect the liquid culture of bacteria infected with M13 phage or phagemid (M13 origin) into 1.5 ml clean tubes, centrifuge at 12,000 rpm for 5 minutes.
2. Transfer 800 μl supernatant to a new clean tube, and add 400 μl Buffer MB and mix thoroughly.

   If the volume of supernatant is more or less than 800 μl, the dosage of Buffer MB should be accordingly increased or reduced.

3. Transfer the mixture from step 2 into a spin-column AC (place the spin column to a collection tube), centrifuge at 10,000 rpm for 15 minutes, and discard flow-through.

   The spin-column can accommodate 700 µl mixture. Please repeat step 3 until all is loaded.

4. Add 700 µl buffer WB (**please check if ethanol is added!).** Centrifuge at 12,000 rpm for 30 seconds and discard flow-through.
5. Place spin-column AC back to the collection tube. Centrifuge at 12,000 rpm for 1 minute. Remove the residue ethanol as much as possible.
6. Transfer spin column AC to a new clean centrifuge tube. Add 60 µl buffer EB (preheated in a water bath with 65-70℃) directly onto the silica-membrane. Incubate 1 minute at room temperature. Centrifuge at 13,000 rpm at 1 minute.

   The volume of elution buffer could be adjusted according to needs. Appropriately reduce elution volume can increase concentration. The minimum volume is 50 µl, and less than 50 µl will decrease the elution efficiency and the DNA yield.

7. Store DNA at -20℃ or apply to down-stream reactions.