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M13 Phage Single Stranded Kit

Product Number: DNK2101

Shipping and Storage

1. Buffer MB may precipitate and precipitate at low temperatures. It can be dissolved again in a water bath at 37℃ for a few minutes to restore clarity and transparency. After cooling to room temperature, it can be used.
2. To avoid volatilization, oxidation, and pH changes caused by prolonged exposure of reagents to the air, each solution should be covered tightly in a timely manner after use.

Component

Component	Storage	DNK2101 50 Preps	DNK2102 100 Preps	DNK2103 200 preps
Buffer MB	RT	25 ml	50 ml	100 ml
Buffer WB	RT	15 ml	25 ml	2×25 ml
Buffer EB	RT	10 ml	20 ml	40 ml
Adsorption column AC	RT	50	100	200
Collection tube (2ml)	RT	50	100	200

Description

M13 and other filamentous phage vectors are very useful in library construction and providing single stranded DNA and inducing mutations for sequence sequencing. Centrifuge an appropriate amount of liquid culture infected with M13 filamentous bacteriophages or related phages (M13 source). The single-stranded bacteriophage DNA in the supernatant selectively adsorbs onto the silica matrix membrane in a high dissociation salt state. Then, through a series of rapid rinsing centrifugation steps, impurities such as salt, cell metabolites, and proteins are removed. Finally, the pure bacteriophage single-stranded DNA is eluted from the silica matrix membrane by low salt Buffer EB.

Features

1. No toxic reagents such as phenol are required, and no steps such as ethanol precipitation are required.
2. Time saving, concise, and single sample operation can generally be completed within 10 minutes.
3. High yield, typical production of 800μl M13 filamentous bacteriophage supernatant can extract 3μg of phage single stranded DNA.
4. Multiple column washes ensure high purity, with a typical OD260/0D280 ratio of 1.7 to 1.9. It can be directly used for sequencing, typically with a recognizable reading of up to 650bp.

Application

Suitable for rapid extraction of M13 phage single stranded DNA

Note

1. All centrifugation steps are completed at room temperature using a traditional desktop centrifuge with a speed of up to 13000rpm.
2. Preheat the required water bath to 50℃ for later use before starting the experiment.
3. Buffer MB contains irritating compounds, and latex gloves should be worn during operation to avoid contact with skin, eyes, and clothing. If it gets on the skin or eyes, rinse with plenty of water or physiological saline.

Protocol(Please read the note before the experiment)

For example, extracting bacterial culture supernatant from 800μl phage infection:

Tip: Before the first use, please add the specified amount of anhydrous ethanol to Buffer WB and mix well. After adding, please mark the box with a check mark indicating that ethanol has been added in a timely manner to avoid adding it multiple times!

1. Divide the liquid culture infected with M13 filamentous phages or related phages (M13 source) into 1.5ml centrifuge tubes and centrifuge at 12000 rpm for 5 minutes to precipitate the bacterial cells.
2. Carefully transfer 800μl of supernatant into a new 1.5ml centrifuge tube, add 400μl of Buffer MB, and mix well.
   If the amount of Buffer MB used is greater than or less than 800μl, it needs to be increased or decreased proportionally.
3. Add the above mixture to an adsorption column AC, centrifuge at 13000npm for 15 seconds (with the adsorption column placed in the collection tube), and discard the waste liquid in the collection tube.
   The adsorption column can only accommodate up to about 700μl of mixture at a time, so it is necessary to add the mixture to the adsorption column in stages and repeat step 3.
4. Add 700μl of Buffer WB (please check if anhydrous ethanol has been added first!) and centrifuge at 12000rpm for 30 seconds to discard the waste liquid.
5. Put the adsorption column AC back into the empty collection tube, centrifuge at 13000rpm for 2 minutes, and try to remove Buffer WB as much as possible to avoid residual ethanol in Buffer WB inhibiting downstream reactions.
6. Take out the adsorption column AC and place it in a clean centrifuge tube. Add 60μl of Buffer EB (pre heated in a 50 ℃ water bath) to the middle of the adsorption membrane. Let it stand at room temperature for 1 minute and centrifuge at 12000 rpm for 1 minute. If you want to obtain a large amount of DNA, you can add the obtained solution back to the centrifuge adsorption column and centrifuge at 10000 rpm for 1 minute.
   The larger the elution volume, the higher the elution efficiency. If a higher DNA concentration is required, the elution volume can be appropriately reduced, but the minimum volume should not be less than 40μl. If the volume is too small, the DNA elution efficiency will be reduced, reducing DNA production.
7. DNA can be stored at 2-8℃, and if it needs to be stored for a long time, it can be placed at -20℃.

Appendix (Preparation process for culture supernatant of M13 phage infected bacteria):

The following is an example to illustrate the preparation process of M13 phage infection bacterial culture supernatant. For detailed information on M13 phage (or M13 derived phage) culture and supernatant preparation, please refer to the second edition of Molecular Cloning.

1. Shake at 37 ℃ overnight to cultivate suitable host bacteria (such as JM109).
2. Inoculate fresh LB culture medium with 6% overnight culture bacteria and shake at 37 ℃ for one hour.
3. According to the concentration (titer) of the storage solution of M13 bacteriophage, add bacteriophages in a ratio of 0.5-1.5% (V/V) to infect the host bacteria. Shake and cultivate at 37 ℃ for 5-6 hours.
4. Divide the liquid culture infected with M13 filamentous phage or related phage (M13 source) into 1.5ml centrifuge tubes and centrifuge at 12000 rpm for 5 minutes to precipitate the bacterial cells.
5. **Optional steps:** Carefully transfer 1ml of supernatant into a new 1.5ml centrifuge tube, repeat step 4 and centrifuge for 5 minutes.
   This step helps to remove trace amounts of host bacterial RNA or DNA remaining in the supernatant.
6. Carefully transfer 800μl of supernatant into a new 1.5ml centrifuge tube.
7. Now you can extract phage single stranded DNA according to the operating steps.