

MEBEP TECH(HK) Co., Limited

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PCR Purification Kit

Product Number: DRK0201

Shipping and Storage

- 1. All solutions should be clear. If the ambient temperature is low, the solution may form precipitates and should not be used directly. It can be heated in a 37°C water bath for a few minutes to restore clarity. It should be restored to room temperature before use.
- 2. Storage at low temperatures (4°C or -20°C) can cause solution precipitation and affect the effectiveness of use. Therefore, transportation and storage are carried out at room temperature (15°C-25°C).
- 3. To avoid volatilization, oxidation, and pH changes caused by prolonged exposure of reagents to the air, each solution should be covered tightly after use.

Components

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Component	Storage	DRK0201	DRK0202	DRK0203
		50Preps	100 Preps	200Preps
Balance Buffer	RT	5ml	10ml	20ml
Buffer BB	RT	25ml	50ml	100ml
Buffer WB	RT	13ml	25ml	50ml
Buffer EB	RT	10ml	10ml	15ml
Adsorption column EC	RT	50	100	200
Collection tube(2ml)	RT	50	100	200

Description

In the presence of high dissociation salts, DNA fragments selectively adsorb onto the silica matrix membrane inside the centrifuge column. Then, a series of rapid rinsing and centrifugation steps are carried out to remove impurities such as primers, nucleotides, proteins, enzymes, etc. from the protein solution and Buffer WB. Finally, pure DNA is eluted from the silica matrix membrane using a low salt, high pH elution buffer.

Features

- The silicon matrix membranes inside the centrifugal adsorption column are all made by imported world-renowned companies, with minimal differences in adsorption capacity between columns and good repeatability. Overcoming the drawback of unstable membrane quality in domestic reagent kits.
- 2. We used high-quality binding solution, which does not contain traditional binding solutions such as sodium iodide and perchlorate, and does not inhibit downstream reactions such as enzymatic cleavage and cloning after recovery.
- 3. Buffer BB is modulated with phenol red to a yellow color, which facilitates monitoring of pH changes and achieves optimal binding effect, greatly improving recovery efficiency.
- 4. It is fast and convenient, and does not require the use of toxic reagents such as phenol and chloroform, nor does it require ethanol precipitation.

Application

Suitable for purification and recovery of PCR reaction products, enzyme cleaved DNA fragments, probe labeling, DNA sample concentration, etc.

Note

1. All centrifugation steps are completed at room temperature using a traditional desktop centrifuge with a rotational speed of up

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to 13000rpm.

- 2. Buffer BB contains irritating compounds, and latex gloves should be worn during operation to avoid contact with skin, eyes, and clothing. If it comes into contact with the skin or eyes, immediately rinse with plenty of water or physiological saline.
- 3. The recovered and purified DNA fragments are generally between 100bp and 40kb, and the recovery efficiency of long and short fragments rapidly decreases.
- 4. The amount of recovered DNA is related to the amount of starting DNA, elution volume, and DNA fragment size. Generally, DNA fragments ranging from 1-15μg to 100bp-5kb can have a recovery rate of up to 95%.
- 5. **Buffer EB does not contain chelating agent EDTA** and does not affect downstream enzyme digestion, connection, and other reactions. Water can also be used for elution, but it should be ensured that the pH is greater than 7.5, as low pH affects elution efficiency. After washing with water, the DNA fragments should be stored at -20°C. If DNA fragments need to be stored for a long time, they can be eluted with TE buffer (10mM Tris HCl, 1mM EDTA, pH 8.0). However, EDTA may affect downstream enzyme digestion reactions and can be appropriately diluted when used.

Protocol

Note:Before using it for the first time, please add the specified amount of anhydrous ethanol to the Buffer WB. After adding it, please mark it as ethanol added in a timely manner to avoid adding it multiple times!

- 1. Add Buffer BB to PCR amplification or enzyme digestion products in a ratio of 5:1 (Buffer BB: sample) (minimum 10μl: 50μl) and mix thoroughly. (For example, after 50μl PCR amplification or enzyme digestion, add the product to 250μl Buffer BB and mix thoroughly).
- 2. Column equilibrium: Add 100μl Balance Buffer to the adsorption column EC, centrifuge at 12000 rpm for 1 minute, discard the filtrate, and set aside for later use.
 - Note:Balance Buffer can enhance the adsorption capacity of silica gel membrane for nucleic acids. Please use the adsorption column processed on the same day.
- 3. Add the solution obtained in the previous step to the adsorption column EC (place the adsorption column in the collection tube), let it stand at room temperature for 1 minute, centrifuge at 12000 rpm for 1 minute, and discard the filtrate.
 - Note:1)If the total volume exceeds 750μl, the solution can be added twice to the same adsorption column EC.
 - 2)After mixing the filtered Buffer BB with the remaining strong alkaline Balance Buffer in the collection tube, Buffer BB may change from yellow to orange red or even purple, which is a normal color change under alkaline conditions of phenol red pH indicator.
- 4. Add 600μl Buffer WB (please check if anhydrous ethanol has been added first!), centrifuge at 12000 rpm for 30 seconds, and discard the filtrate. Add 600μl Buffer WB and rinse again, discard the filtrate.
- 5. Return the adsorption column to the empty collection tube and centrifuge at 12000 rpm for 2 minutes to remove Buffer WB as much as possible to prevent residual ethanol in Buffer WB from inhibiting downstream reactions.
- 6. Remove the adsorption column and place it in a clean centrifuge tube. Add 50μl Buffer EB (Buffer EB can be preheated in a water bath at 80°C-90°C to increase yield) to the middle of the adsorption membrane. Let it stand at room temperature for 2 minutes, centrifuge at 12000 rpm for 1 minute, and discard the adsorption column.
 - Note:1)Recommendation: To increase the efficiency of DNA recovery, the obtained solution can be re added to the centrifugal adsorption column, left at room temperature for 1 minute, and centrifuged at 12000 rpm for 1 minute. Washing twice can increase the concentration by about 10%.
 - 2)The larger the elution volume, the higher the elution efficiency. If a higher DNA concentration is required, the elution volume can be appropriately reduced. However, it should be noted that a smaller volume can reduce DNA elution efficiency and yield (the minimum should not be less than 25µl).