

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	30ml	60ml	100ml
Buffer WB	RT	13ml	25ml	50ml
Buffer EB	RT	10ml	15ml	20ml
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.

About the use of Balance Buffer

1. Description

During the long-term storage of nucleic acid adsorption silica gel membrane columns, they react with charges/dust in the air and affect their nucleic acid binding ability. After pre-treatment with equilibrium solution, the silica gel column can greatly reduce the hydrophobic groups of the silica gel membrane in the column and improve the binding ability of nucleic acids. Thus improving the recovery efficiency or yield of silicone columns. The equilibrium solution is a strong alkaline solution. If accidentally touched, please clean it with a large amount of tap water. After use, the bottle cap needs to be tightly closed to avoid contact with air. Store at room temperature. During storage, there may be precipitation formation. Please heat to 37°C to completely eliminate the precipitation.

2. Protocol

Take a new silica gel membrane adsorption column and install it in a collection tube, and suck 100µl of equilibrium solution into the column. Centrifuge at 13000rpm for 1 minute, discard the waste liquid from the collection tube, and reposition the adsorption column in the collection tube. At this point, the balance liquid pre-treatment column is completed. Follow the subsequent operating steps.

Protocol

Note: Reminder: 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!

- Add 500μl of Buffer BB to every 100μl of PCR amplification or enzyme digestion system, and mix thoroughly. (If the initial system is less than 100μl, please adjust to 100μl with double distilled water in advance).
 - Balance Buffer pre-treatment adsorption column: The use of Balance Buffer pre-treatment of silicone membrane adsorption column is a necessary step. For specific methods, please refer to the previous section "About the Use of Balance Buffer"
- Add the solution obtained from the previous step to the adsorption column EC (the adsorption column is placed in the
 collection tube), place at room temperature for 1 minute at 12000 rpm, centrifuge for 30-60 seconds, and discard the waste
 liquid in the collection tube.
 - Note: After combining the filtered Buffer BB with the residual strong alkaline equilibrium solution in the collection tube, the sol solution may change from yellow to orange red or even purple, which is the normal color change of the phenol red pH indicator under alkaline conditions.
- 3. Add 600µl of Buffer WB (**please check if anhydrous ethanol has been added first!**) and centrifuge at 12,00rpm for 30 seconds to discard the waste liquid.
- 4. Add 600μl Buffer WB and centrifuge at 12000 rpm for 30 seconds, discard the waste liquid.
- 5. Return the adsorption column EC to the empty collection tube, centrifuge at 12000 rpm for 2 minutes, and try to remove Buffer WB as much as possible to prevent residual ethanol from inhibiting downstream reactions in Buffer WB.



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6. Take out the adsorption column EC and place it in a clean centrifuge tube. Add 50μl of Buffer EB in the middle of the adsorption membrane (Buffer EB is better heated in a water bath at 65-70°C beforehand), and leave it at room temperature for 2 minutes at 12000 rpm for 1 minute. If a large amount of DNA is required, the obtained solution can be re added to the adsorption column and centrifuged for 1 minute.

Note: The larger the elution volume, the higher the elution efficiency. If a high DNA concentration is required, the elution volume can be appropriately reduced, but the minimum volume should not be less than 25μ l. If the volume is too small, it will reduce the DNA elution efficiency and reduce production.