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# Quick PAGE Extraction Kit

**Product Number: DRK0501**

## Shipping and Storage

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.

## Components

| Component | Storage | DRK0501<br>50 Preps |
|---|---|---|
| Balance Buffer | RT | 5ml |
| Buffer CB | RT | 100ml |
| Buffer DS | RT | 30ml |
| Buffer WB | RT | 15 ml |
| Buffer EB | RT | 15 ml |
| Adsorption column EC | RT | 50 |
| Collection tube (2ml) | RT | 50 |

## Description

This kit uses a new silicon-based plasma membrane centrifuge column and a special buffer system, which can easily and efficiently recover 20bp-500bp short DNA fragments from polyacrylamide gel, and the recovery efficiency can be as high as 85%. And it can remove impurities to the maximum extent, obtain high-purity DNA, and the obtained DNA can be directly used for subsequent molecular biology experiments such as enzyme digestion, ligation, sequencing, etc.

## Features

1. Widely applicable, it can recover single or double stranded DNA of 20bp-2kb.
2. High quality Buffer CB was used, which does not contain traditional Buffer CB's sodium iodide and perchlorate, and does not inhibit downstream reactions such as enzyme digestion and ligation cloning after recovery.
3. The unique formula ensures that the recovery efficiency of this reagent kit is greatly improved compared to ordinary reagent kits.
4. Fast and convenient centrifugal column type, without the use of toxic reagents such as phenol and chloroform, and without the need for ethanol precipitation.

## Note

1. All centrifugation steps are completed at room temperature using a traditional desktop centrifuge with a speed of up to 13000rpm.
2. Buffer CB 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, immediately rinse with plenty of water or physiological saline.
3. It is best to use a new electrophoresis buffer during electrophoresis to avoid affecting the electrophoresis and recovery results.
4. When cutting glue, the UV irradiation time should be as short as possible to avoid damage to DNA. Alternatively, visible light dyes can be used to dye and cut the gel under visible light to avoid UV damage to DNA.
5. The recovery rate was related to fragment size, gel concentration, initial DNA amount and elution volume.

## 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℃ 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

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

1. Cut the PAGE gel containing DNA fragments (about 100mg, cut off as much as possible of the excess glue, otherwise the recovery efficiency will be affected), put it into a 1.5mL centrifuge tube, and use the pipette gun head to smash it as much as possible (it is better to burn the mouth of the gun head in an alcohol lamp first, and then use it to smash it), the finer the better.
2. Add 1-2 times the volume of Buffer DS to the gel (for example, add 100-200μl Buffer DS to 100mg gel), warm bath at 55℃ for 30 minutes to 2 hours, during which vortex vibration and mixing are performed every 15 minutes to promote the diffusion of DNA in the gel to the solution.

   **Generally speaking, the larger the recovered fragment, the longer it takes for DNA diffusion. A DNA fragment of about 100bp can be incubated for 30 minutes (longer time does not affect the recovery effect); If DNA fragments ranging from 500bp to 1000bp are recovered, the warm bath time can be extended by 3-5 hours. You can also take a warm bath at 37 ℃ for 12-16 hours overnight.**
3. Centrifuge at 12000 rpm for 5 minutes. Carefully transfer the supernatant into a new centrifuge tube. Record the volume of the supernatant.
4. Add buffer CB with 9 times the volume of supernatant and mix well.

   **When recycling fragments>100bp, add 5 times the volume of buffer CB to the supernatant.**
5. Add the solution obtained from the previous step to the adsorption column EC (the adsorption column is placed in the collection tube), let it stand at room temperature for 1 minute, centrifuge at 12000 rpm for 30-60 seconds, and discard the waste liquid in the collection tube.

   **The maximum volume of the adsorption column is 750μl. If the solution volume is greater than 750μl, it can be added in batches.**
6. Add 600μl Buffer WB (please check if anhydrous ethanol has been added first!), centrifuge at 12000rpm for 30 seconds, and discard the waste liquid.
7. Repeat step 6 once.
8. Put the adsorption column EC 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.
9. Take out the adsorption column EC and place it in a clean centrifuge tube. Add 30-50μl Buffer EB to the middle of the adsorption membrane and let it stand at room temperature for 2 minutes. Centrifuge at 12000 rpm for 1 minute. If a higher concentration of nucleic acid is required, the obtained solution can be re added to the adsorption column and centrifuged for 1 minute.

   **The larger the elution volume, the higher the elution efficiency. If a high nucleic acid concentration is required, the elution volume can be appropriately reduced, but the minimum volume should not be less than 25μl. If the volume is too small, it will reduce the efficiency of nucleic acid elution and reduce production.**