

Mutifunction DNA Recover Kit

Product Number: DRK0301

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. Storing at low temperatures (4°C or -20°C) can cause solution precipitation, affecting 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 in a timely manner after use.

Components

Component	Storage	DRK0301	DRK0302	DRK0303
		50 Preps	100 Preps	200 Preps
Balance Buffer	RT	5ml	10ml	20ml
Buffer HB	RT	20ml	40ml	80ml
Buffer WB	RT	13 ml	25 ml	50 ml
Buffer EB	RT	10 ml	15 ml	15 ml
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. Through a series of rapid rinsing centrifugation steps, Buffer WB removes impurities such as primers, nucleotides, proteins, enzymes, etc. Finally, low salt, high pH Buffer EB elutes pure DNA from the silica matrix membrane.

Features

1. The silicon matrix membranes inside the centrifugal adsorption column are all made of specially designed adsorption membranes, with minimal differences in adsorption capacity between columns and good repeatability.
2. Buffer HB, prepared with high-quality guanidine isothiocyanate, does not contain traditional sol solutions of sodium iodide and perchlorate, and does not inhibit downstream reactions such as enzyme digestion and cloning after recovery.
3. By using high concentration buffer HB, equal volume sol can be obtained, reducing operational steps.
4. The unique Buffer HB formula unifies the functions of sol-gel and binding, so a single kit can be used for various situations such as agarose DNA recovery, PCR product cleaning and purification, enzyme digestion product purification and recovery, saving the cost of purchasing multiple kits.
5. Buffer HB is modulated to a yellow color, which facilitates observation of sol effect and monitoring of pH changes to achieve optimal binding effect, greatly improving recovery efficiency.
6. The improved Buffer HB formula greatly enhances buffering capacity and stability, allowing for pH buffering within the optimal binding range even with significant sample changes.
7. Fast and convenient, without the need for toxic reagents such as phenol and chloroform, and without the need for ethanol precipitation.

Application

It is suitable for DNA recovery from agarose gel, purification and recovery of PCR reaction products, purification and recovery

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of DNA fragments from enzyme digestion products, purification and recovery after probe labeling, DNA sample concentration, etc.

Note

1. Buffer HB and Balance Buffer contain irritating compounds. When operating, wear latex gloves 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.
2. The recovered and purified DNA fragments are generally between 100bp and 40kb, and the recovery efficiency of long and short fragments rapidly decreases.
3. The amount of recovered DNA is related to the amount of starting DNA, elution volume, and DNA fragment size. Generally 1-15 μ g. A DNA fragment of 100 bp-5 kb can achieve a recovery rate of up to 85% -95%.
4. When cutting and recycling, UV light observation can damage DNA fragments. Low energy long wave ultraviolet light should be used as much as possible, and the processing time under ultraviolet light should be shortened as much as possible.
5. Buffer EB does not contain chelating agent EDTA and does not affect downstream enzyme cleavage, linking, 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 can affect elution efficiency. Wash with water, 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), but EDTA may affect downstream enzyme digestion reactions and can be diluted appropriately when used.

Use of balance buffer

1. Description

During the long-term placement of the nucleic acid adsorption silica gel membrane column, it reacts with the charge/dust in the air, affecting its nucleic acid binding ability. After pre-treatment with Balance Buffer, the hydrophobic groups of the silica gel membrane in the column can be greatly reduced, improving the binding ability of nucleic acids. Thereby improving the recovery efficiency or yield of silicone columns. Balance Buffer is a highly alkaline solution. If accidentally touched, please clean with plenty of tap water. After use, it is necessary to tighten the bottle cap to avoid contact with air. Store at room temperature. During storage, precipitation may occur. Please heat to 37°C to completely eliminate the precipitation.

2. Protocol

Take a new silicone membrane adsorption column and place it in a collection tube. Take 100 μ l of Balance Buffer and transfer it into the column. Centrifuge at 13000rpm for 1 minute, discard the waste liquid in the collection tube, and place the adsorption column back into the collection tube. At this point, the Balance Buffer has completed preprocessing the columns. 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. Agarose gel DNA recovery:

- 1.1. Under the long wave ultraviolet light or blue light, use a clean blade to cut off the DNA strips to be recovered, and try to cut the DNA free gel, so as to get the smaller the gel volume, the better.
- 1.2. Put the cut gel containing DNA strips into a 1.5ml centrifuge tube and weigh it.

First weigh an empty 1.5ml centrifuge tube, then put in a gel block and weigh again. Subtract the two weights to get the weight of gel.

- 1.3. 100 μ l Buffer HB is added to every 100 mg of 1% agarose gel.

For high concentration agarose gel, the addition amount of Buffer HB should be increased in equal proportion. To simplify the experiment, it is recommended to uniformly add 400 μ l of Buffer HB.

- 1.4. Place in a 56°C water bath for 5-10 minutes (or until the glue is completely dissolved). Vortex oscillation every 2-3 minutes helps accelerate dissolution.

Optional, generally not required: For fragments below 400bp, it is recommended to add 0.3 times the volume of

isopropanol to improve the recovery rate.

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 "Use of balance buffer"

1.5. 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.

After mixing the filtered Buffer HB with the remaining strong alkaline Balance Buffer in the collection tube, the Buffer HB 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.

1.6. Add 600µl Buffer WB (please check if anhydrous ethanol has been added first!), centrifuge at 12000rpm for 30 seconds, and discard the waste liquid.

1.7. Repeat step 1.6 once.

1.8. Put the adsorption column EC back into the empty collection tube, centrifuge at 12000 rpm for 2 minutes, and try to remove Buffer WB as much as possible to avoid residual ethanol in Buffer WB inhibiting downstream reactions.

1.9. Take out the adsorption column EC and place it in a clean centrifuge tube. Add 50µl Buffer EB to the middle of the adsorption membrane. Leave at room temperature for 2 minutes and centrifuge 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.

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 25µl. If the volume is too small, it will reduce the DNA elution efficiency and yield. When the initial sample is small, heating the Buffer EB in a water bath at 80-100°C beforehand can improve the elution efficiency.

2. Purification of DNA using PCR products or enzyme cleaved fragments:

2.1. Estimate the volume of PCR reaction solution or enzyme digestion reaction solution and add 3 times the volume of Buffer HB to it. Mix thoroughly (without removing paraffin or mineral oil).

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"

2.2. 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.

2.3. This step is completely consistent with the steps 1.6-1.9 of DNA recovery from agarose gel. Please refer to steps 1.6-1.9 of DNA recovery from agarose gel.