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Universal DNA Purification/Concentrate Micro Kit

Product Number: DRK0601

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℃ water bath for a few minutes to restore clarity. It should be restored to room temperature before use.
2. Storing at low temperatures (4℃ or -20℃) can cause solution precipitation, affecting the effectiveness of use. Therefore, transportation and storage are carried out at room temperature (15℃ -25℃).
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	DRK0601 100rxns	DRK0602 200rxns
Balance Buffer	RT	5ml	10ml
Buffer DB	RT	50ml	100ml
Buffer WB	RT	13 ml	25ml
Buffer EB	RT	10 ml	20ml
Ultratrace DNA centrifuge column	RT	100	200
Collection tube (2ml)	RT	100	200

Description

This product is a specialized reagent kit for ultra trace DNA concentration and recovery. It is applicable to the recovery of agarose gel DNA of trace DNA, purification and recovery of PCR reaction products, purification and recovery of DNA fragments of enzyme digestion products, purification and recovery after probe labeling, DNA sample concentration, etc.

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

Features

1. The special washer free centrifugal column design ensures no liquid residue and contamination after centrifugation. Ensuring high purity of recycled DNA.
2. The special design of a 5ug centrifuge column for trace amounts can achieve a minimum elution of 5ul, ensuring a high concentration of recovered DNA.
3. High quality Buffer DB was used, which 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.
4. The unique Buffer DB 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. The Buffer DB has been modulated to a yellow color, making it easy to observe the sol effect and monitor pH changes to achieve the best binding effect, greatly improving the recovery efficiency.
6. The improved Buffer DB 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.

Note

1. The maximum adsorption capacity of the ultra micro centrifugal column is 5ug, exceeding the range may lead to poor performance.
2. The recovered and purified DNA fragments are generally between 50bp and 40kb, but within this range, the recovery efficiency rapidly decreases as the fragment length becomes too long or too short.
3. The amount of recovered DNA is related to the amount of starting DNA, elution volume, and DNA fragment size. A typical 5ug, 100bp-5kb DNA fragment can achieve a clean recovery rate of up to 85% -95% for PCR products. If the expected fragment is too long, too short, and the recovery rate is low, small volume elution (minimum not less than 5ul) can be chosen to increase the concentration.
4. When cutting and recycling, UV light observation may cause damage to 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 ℃. 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.

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, use a clean blade to cut off the DNA strips to be recovered, and try to cut off the DNA free gel 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. Add 3 times the volume of Buffer DB.

If the gel weighs 100mg and its volume can be regarded as 100μl, then add 300μl of sol solution.

If the gel concentration is greater than 2%, add 6 times the volume of sol solution.

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

1.5. **Optional, generally unnecessary:** add 150μl isopropyl alcohol per 100mg of initial gel weight, shake and mix well.

Sometimes adding isopropanol can improve the recovery rate, do not centrifuge after adding. When recovering fragments larger than 4Kb, not adding isopropanol may sometimes reduce the recovery efficiency.

1.6. Column balance: Add 50μl Balance Buffer to the ultramicro DNA centrifuge column, centrifuge at 12000 rpm for 1 minute, discard the filtrate, and set aside for later use.

Balance Buffer can enhance the adsorption capacity of silica gel membrane for nucleic acids. Please use the adsorption column processed on the same day.

1.7. Add the solution obtained from the previous step to a micro centrifuge column (with the adsorption column placed in the collection tube), let it stand at room temperature for 1 minute, centrifuge at 12000 rpm for 30 seconds, and discard the waste liquid in the collection tube.

If the total volume exceeds 750μl, the solution can be added twice to the same micro centrifuge column.

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

Buffer DB 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.

1.8. Add 300µl Buffer WB (please check if anhydrous ethanol has been added first!), centrifuge at 12000rpm for 30 seconds, and discard the waste liquid.

1.9. Add 300µl Buffer WB and centrifuge at 12000rpm for 2 minutes. Carefully remove the dried micro centrifuge column and place it in a clean centrifuge tube.

This step combines rinsing and air drying centrifugal columns into one. When removing the micro centrifuge column, be careful not to let the lower edge of the centrifuge column touch the Buffer WB waste liquid in the collection tube. To prevent residual ethanol in Buffer WB from inhibiting downstream reactions.

1.10. **Be careful to align the pipette tip with the middle part of the micro centrifuge column adsorption membrane (do not add it to the tube wall).** Add 10µl (5µl-25µl) of Buffer EB (which is better heated in a 65-70 ℃ water bath beforehand), leave at room temperature for 1 minute, and centrifuge at 12000rpm 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 5µl. If the volume is too small, it will reduce the DNA elution efficiency and yield.

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

2.1. Add 100µl Buffer DB to every 20µl PCR amplification or enzyme digestion system and mix thoroughly.

Note: Processing sample volume: Buffer DB volume=1:5

2.2. Column balance: Add 50µl Balance Buffer to the ultramicro DNA centrifuge column, centrifuge at 12000 rpm for 1 minute, discard the filtrate, and set aside for later use.

Balance Buffer can enhance the adsorption capacity of silica gel membrane for nucleic acids. Please use the adsorption column processed on the same day.

2.3. Add the solution obtained from the previous step to a micro centrifuge column (with the adsorption column placed in the collection tube), let it stand at room temperature for 1 minute, centrifuge at 12000 rpm for 30 seconds, and discard the waste liquid in the collection tube.

2.4. From this step, it is completely consistent with the operation steps 1.8-1.10 of DNA recovery from agarose gel. Please refer to the operation steps 1.8-1.10 of DNA recovery from agarose gel.