Note:1) In order to increase the amount of DNA recovered, the solution obtained can be added back to column, and leave it at room temperature for 2 min, and centrifuge at 13,000 rpm for 1 min.
2) The elution volume should not be less than 30 μL. Too little volume will affect recovery efficiency.
3) When DNA fragments are larger than 10 kb, Buffer EB should be preheated in a 50°C water bath to increase recovery efficiency.

Note: This kit is also suitable for the purification and recovery of PCR products. Add an equal volume of Buffer PG to the PCR product and mix thoroughly (for recovery of small fragments less than 150 bp, the volume of the solution can be increased to 3 times to increase the recovery rate). Follow the step 5 above for subsequent operations.

Version: 03/2024

Gel Extraction Kit

Cat. No. : CW2302S (50 preps)
CW2302M (200 preps)

Storage Condition : Room temperature (15-30°C)

Components

Component	CW2302S (50 preps)	CW2302M (200 preps)
Buffer PG	25 mL	100 mL
Buffer PS	15 mL	60 mL
Buffer PW (concentrate)	10 mL	50 mL
Buffer EB	10 mL	30 mL
Spin Columns DM with Collection Tubes	50	200

Introduction

The kit uses new silicon matrix membrane technology and reagent formula, and can recover and purify 100 bp-10 kb DNA fragments from ordinary or low melting point agarose gel quickly through a unique centrifugal adsorption column combined with DNA - washing - elution steps. The rate of gel dissolving is fast, and the recovery efficiency is high. The solution contains pH indicator, which can be used to judge whether the gel recovery is in the best state according to the color. Each adsorption column can adsorb up to 10 μg of DNA and effectively remove primers, enzymes, mineral oil, agarose and other impurities. The purified DNA has high purity, concentration and integrity, and can be directly used in molecular biology experiments such as sequencing, ligation and transformation, labeling, in vitro transcription.

Reagents to be Supplied by user

100% ethanol, Isopropanol.

Precautions

1. 100% ethanol should be added to the Buffer PW before the first use according to the instructions on the reagent bottle label.
2. Please check the Buffer PG before use. If crystallization or precipitation occurs, it can be placed in a 37℃ water bath for 3-5 minutes to become clear.
3. It is better to use new running buffer during electrophoresis. If the downstream experiment requires a higher level, please try to use TAE buffer.
4. When cutting the gel, the exposure time to UV should be as short as possible to avoid DNA damage.
5. The recovery efficiency is related to the initial amount of DNA and the elution volume. The smaller the initial amount, the smaller the elution volume and the lower the recovery efficiency.
6. Preheat the water bath to 50℃.
7. Buffer PG contains pH indicator. When the pH is ≤ 7.5, the color of the solution is yellow. At this time, the DNA can effectively bind with the membrane. When the pH is too high, the color of the solution turns orange and purple and needs to be adjusted.
8. All centrifuge steps can be done at room temperature.

Protocol

1. Cut the single DNA band from the agarose gel; Place it in a clean centrifuge tube (self-prepared), and weight the gel (weight the centrifuge tube first)

Note: If the volume of the gel is too big, cut it into pieces.

2. Add 1 times the volume of Buffer PG to the tube (eg, for a 100 mg gel, add 100 μL Buffer PG).
3. Incubate the tube in a 50℃ water bath and gently invert the tube every 2-3 min until the solution is yellow to ensure that the gel is fully dissolved. If there is undissolved gel, add more Buffer PG and wait for a few more minutes until the gel is completely dissolved.

   Note:1) After the gel is completely melted, the gel solution will be yellow, and you can proceed to the next step; if the gel solution is orange-red or purple, you can add 10-30 μL of 3M sodium acetate (pH 5.0) to the gel solution to adjust the color of the solution to After turning yellow, proceed to the next step.

   2) After the gel block is completely dissolved, it is best to lower the temperature of the gel solution to room temperature before transferring it to the adsorption column. The adsorption column has poor ability to bind DNA at higher temperatures.
4. (Optional) If the DNA fragment is < 300 bp, add 1⁄2 volume of isopropanol and mix by upside down (eg. if the gel is 100 mg, add 50 μL of isopropanol).
5. Column balance: Add 200 μL Buffer PS to Spin Columns DM with Collection Tubes, centrifuge 13,000 rpm (~16,200 ×g) for 1 min. Discard the waste liquid, and put the adsorption column back into the tube.
6. Transfer the solution obtained in step 3 or 4 to a column with collection tube, leave it at room temperature for 2 min, and centrifuge at 13,000 rpm for 1 min. Discard the waste liquid, and put the adsorption column back into the tube.

   Note: The maximum volume of the column is 750 μL. If the sample volume is more than 750 μL, it can be added in batches.
7. Add 450 μL Buffer PW (please check whether 100% ethanol has been added before use) to the adsorption column, centrifuge with 13,000 rpm for 1 min. Discard the waste liquid, and put the adsorption column back into the tube.

   Note: If the purified DNA is used for salt sensitivity tests (such as blunt-end ligation test or direct sequencing), it is recommended to add Buffer PW and leave it at rest for 2-5 min before centrifugation.
8. Repeat step 7.
9. Centrifuge at 13,000 rpm for 1 min and discard the waste from the collection tube.

   Note: The purpose of this step is to remove the residual ethanol in the adsorption column. The residual ethanol will affect subsequent enzymatic reactions (enzyme digestion, PCR, etc.).
10. Place the column in a new 1.5 mL centrifuge tube (self- prepared) and add 50 μL of Buffer EB in the middle of the membrane and allow it to stand at room temperature for 2 min. Centrifuge at 13,000 rpm for 1 min and collect the DNA solution. Store the DNA at -20℃.