

Agarose Gel DNA Recovery Kit

Project number: G665990

Storage conditions: room temperature.

Product Content:

Component	G66599020 0preps
BufferPG	100ml
BufferPS	60ml
BufferPW (concentrate)	50ml
BufferEB	30ml
SpinColumnsDM withCollectionTubes	200

Product Introduction

This kit adopts the new silicon matrix membrane technology and reagent formulation, through the unique centrifugal adsorption column can be quickly combined with the DNA-washing-elution step can be recovered from ordinary or low melting point agarose gel purification of DNA fragments of 100bp-10kb, the speed of lysis, high recovery rate. The lysate contains a pH indicator, which can be used to determine whether the lysate recovery is optimal according to the color. Each adsorption column can adsorb up to 10 μ g of DNA, while effectively removing primers, enzymes, mineral oil, agarose and other impurities. The purified and recovered DNA is of high purity and concentration, with good integrity, and can be directly used in molecular biology experiments such as sequencing, ligation and transformation, labeling, and in vitro transcription.

Self-contained reagents: anhydrous ethanol, isopropanol.

Pre-experiment Preparation and Important Notes

1. Anhydrous ethanol should be added to BufferPW according to the instructions on the label of the reagent bottle before first use.
2. Please check BufferPG before use. If crystallization or precipitation occurs, it can be placed in a 37°C water bath for 3-5 minutes to restore clarification.
3. When electrophoresis, it is better to use new electrophoresis buffer to avoid affecting the electrophoresis and recovery effect; the next step of the experiment is more demanding, please try to use TAE electrophoresis buffer.
4. When cutting glue, ultraviolet irradiation time should be as short as possible

to avoid damage to DNA.

5. The recovery is related to the initial amount of DNA and the elution volume; the lower the initial amount and the lower the elution volume, the lower the recovery.
6. Preheat the water bath to 50° C.
7. BufferPG contains pH indicator, when $\text{pH} \leq 7.5$ the color of the solution is yellow, when the DNA can effectively bind to the membrane, when the pH value is high the color of the solution changes to orange and purple, need to be adjusted.
8. All centrifugation steps can be performed at room temperature.

procedure

1. Cut the single-purpose DNA strip from the agarose gel (try to remove as much excess as possible) and place it in a clean centrifuge tube (supplied), weigh and calculate the weight of the gel (record the weight of the tube in advance).

Note: If the glue block is too large, cut the block into pieces.

2. Add 1x volume of BufferPG to the gel block (if the gel weighs 100 mg, the volume can be considered as 100 μl , and so on).

3. Warm up in a 50° C water bath, during which the tube is gently tilted up and down every 2-3 minutes to ensure that the colloid is fully dissolved, until the solution is yellow. If there is still undissolved glue block, you can add some more gel solution or continue to leave a few minutes until the glue block is completely dissolved.

Note: 1) The gel solution is yellow after the gel is completely melted, and the subsequent operation can be carried out; if the gel solution is orange-red or purple, 10-30 μl of 3M sodium acetate (pH5.0) can be added to the gel solution to adjust the color of the solution to yellow before carrying out the subsequent operation.

- (2) It is better to reduce the temperature of the gel solution to room temperature before loading the column after the gel block is completely dissolved; the adsorption column has a weaker ability to bind DNA at higher temperatures.

4. (Optional step) When the recovered fragment is <300bp, 1/2 gel volume of isopropanol should be added, mixing up and down (if the gel weighs 100mg, add 50 μl of isopropanol).

5. Column equilibration: Add 200 μl BufferPS to the adsorbent column (SpinColumnsDM) that has been loaded into the collection tube, centrifuge at 13,000 rpm ($\sim 16,200 \times g$) for 1 min, pour off the waste liquid in the collection tube, and put the adsorbent column back into the collection tube.

6. Add the solution from step 3 or 4 to the adsorbent column in the collection tube, let stand at room temperature for 2 minutes, centrifuge at 13,000 rpm for 1 minute, pour off the waste liquid from the collection tube, and return the column to the collection tube.

Note: The adsorption column volume is 750 μ l, if the sample volume is larger than 750 μ l can be added in batches.

7. Add 450 μ l of BufferPW to the adsorption column (check that anhydrous ethanol has been added before use), centrifuge the column at 13,000 rpm for 1 minute, pour off the waste liquid in the collection tube, and place the column back into the collection tube.

Note: If purified DNA is used for salt-sensitive experiments (e.g. flat-end ligation or direct sequencing), it is recommended to add BufferPW and let it sit for 2-5 minutes before centrifugation.

8. Repeat step 7.

9. Centrifuge at 13,000 rpm for 1 minute and pour off the waste liquid in the collection tube.

Note: The purpose of this step is to remove residual ethanol from the adsorption column, which can interfere with subsequent enzymatic reactions (digestion, PCR, etc.).