GENCYST

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Gel DNA Purification Kit

Catalog No.	Specification	Storage/Shelf life
EP006-50T	50T	Room temperature /1 year
EP006-200T	200T	Room temperature /1 year

Introduction

This kit can recover high-purity DNA fragments containing no salt or low salt concentration and no impurities such as protein and RNA from various concentrations of agarose gel. The 200 bp-10 kbp DNA fragment has a recovery rate of 80% or more. and single-stranded. double-stranded DNA fragments and circular plasmid DNA can be recovered. The recovered fragment DNA can be directly subjected to enzyme digestion,enzyme ligation,and sequencing reaction.

Kit Components

Component	EP006-50T	EP006-200T
Solution G	60 ml	240 ml
Wash Buffer	60 ml	240 ml
Elution Buffer	5 ml	20 ml
Adsorption column G column	50 set	200 set
User manual	1 copy	1 copy

Before starting

Solution G: If it is not used for a long time after opening. check the pH of Solution G to ensure pH≤ 7.5

Wash Buffer: Add the anhydrous ethanol into the Wash Buffer (labeled on the reagent bottle) before use.

Heating in water bath: The gel step needs to be 5-10 min in a 55 ° C water bath environment. It is recommended to preheat the bath in advance.

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Operation steps

1. Using a clean,sharp cutting blade to cut the target strip after gel electrophoresis and loaded into a 2 ml of colorless,transparent EP tube.

   *Note: Pay attention to weighing the EP tube in advance because the next step is to roughly estimate the gel volume and the amount of the sol buffer by the gel mass.

2. Weigh the mass of the gel and the EP tube and calculate the individual mass of the gel block. The accuracy is 0.01 g

   *Note: Because gel electrophoresis usually contains toxic dyes. it is strongly recommended that you wear gloves and put a weighing paper on when weighing. so as not to pollute the experimental environment and affect your own safety.

3. According to the mass of the gel block,at the ratio of 1G = 1000 µl,adding 3 times the volume of the sol buffer Solution G (for example. 0.1g gel needs to add 300 µl gel buffer) (adding 6 times the sol buffer when gel concentration is more than 2%). 55 ℃ water bath sol 5 - 10 min,until the gel completely dissolved.

   *Note: The refractive index can be observed by shaking the EP tube to determine whether the gel is completely dissolved. It can also be determined by high-speed instantaneous centrifugation to determine whether the gel is completely dissolved. Only when the gel is fully dissolved can the next experiment be carried out. otherwise the recovery rate will be seriously reduced.

4. (Optional) Transfer the EP tube containing the dissolved complete gel to a high-speed centrifuge and centrifuge at 10.000 × g for 30 sec at 4 ° C to remove the residual solution adsorbed on the wall of the centrifuge tube and rapidly cool the mixture to increase the membrane. Maximize recovery rate.
5. Add the completely dissolved gel solution obtained in the previous step to the adsorption column G column provided by the kit (if it can not be added once. it can be added in multiple times),and centrifuge at 10.000 × g for 1 min at room temperature. Discard the waste liquid from the collection tube. If necessary, this step can be repeated once,the recovery rate of nucleic acid at a low concentration is significantly improved.
6. Add 300 µl of Solution G to the adsorption column G. centrifuge at 10.000 × g for 1 min at room temperature,and discard the waste.
7. Add 600 µl Wash Buffer to the G column of the adsorption column. centrifuge at 13.000 × g for 1 min at room temperature,and discard the waste.

8. Repeat step 7.
9. Centrifuge at 13.000 xg for 2 min at room temperature to thoroughly remove the residue of Wash Buffer.
10. Remove the adsorption column G column and put it into a new EP tube. Leave the adsorption column G column open for 2 min at room temperature. If necessary, put it in the air conditioning vent for 1-2 min to completely remove residual ethanol.
11. Add 15-50 µl (recommended amount 30 µl) to the middle of the adsorption column G column. Elution Buffer or ddH2O (the solution after 55 °C water bath dissolves better), let it stand for 5 min to completely dissolve the plasmid, at room temperature,the recovered DNA fragment was obtained by centrifugation at 13.000 x g for 2 min.

    *Note: The recovered DNA can be directly used for gene cloning, amplification, sequencing, enzymatic digestion. etc.

DNA concentration and purity

DNA concentration(µg/ml)=OD_{260} × 50×Dilution factor.

OD_{260}/ OD_{280} is about 1.8-2.0

Attention

The kit is only suitable for the recovery of agarose gel electrophoresis products, but not for the recovery of polyacrylamide gel. Each component in the kit has a certain volatility, please close the cap immediately after use,in order to prevent contamination or volatilization of the reagent and reduce the effect.

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Frequently Questions & Answers

Frequently Questions	Possible Causes	Suggestions
Low recovery or no banding	The gel is not completely dissolved in the sol	When the gel is used, the time is prolonged appropriately, and the mixture is oscillated several times during the period to ensure the dissolution is complete.
	The gel is too large	The gel in each tube should be less than 300mg.
	No absolute ethanol was added to Wash Buffer.	Make sure to add absolute ethanol to the Wash Buffer.
	Improper use of Wash Buffer	Ensure the use of Wash Buffer provided by kits.
	Insufficient elution	Ensure sufficient elution time. Elution Buffer can be preheated at 55 °C before use, direct sequencing or enzymatic digestion is recommended to dissolve in deionized water.
	Electrophoresis buffer PH is too high	Make sure to use fresh buffer when electrophoresing the sample to be tested.
	Too few samples, too low concentration	Increase sample consumption
	Gel is too thin	Increase the thickness of the gel to ensure that DNA does not swim into the pool when electrophoresed.
Recycled products cannot be used for subsequent experiments	Ethanol residue	When the room temperature is low, the drying time can be extended appropriately, or the residual ethanol can be dried before being placed in the air conditioner.
	Salt residue	Make sure the amount of washing liquid and the number of washings are separated from each other by centrifugation.