ELK Biotechnology

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PCR Circle Purification Kit

Catalog No.	Specification	Storage/Shelf life
EP005-50T	50T	Room temperature /1 year
EP005-200T	200T	Room temperature /1 year

Introduction

The kit recovers high-purity DNA fragments containing no salt or low salt and no impurities such as protein or RNA from the PCR reaction system. The 200 bp-10 kb DNA fragment has a recovery rate of more than 80%. and can recover single-stranded,double-stranded DNA fragments and circular plasmid DNA. The recovered fragment DNA can be directly subjected to enzyme digestion. enzyme ligation. and sequencing reaction.

Kit Components

Component	EP005-50T	EP005-200T	Storage
Solution PG	20 ml	80 ml	RT
Wash Buffer	60 ml	240 ml	RT
Elution Buffer	5 ml	20 ml	RT
Adsorption column G column	50 set	200 set	RT
User manual	1 copy	1 copy	RT

Before starting

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

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

Operation steps

1. PCR product : Solution PG according to the ratio of 1: 5 add into Solution PG (For example: add 250 μl Solution PG to 50 μl PCR reaction system) and mix upside down. If the fragment length is less than 500 BP. add Solution PG to 1:6

ratio.

2. (Optional) Transfer the EP tube containing the mixed solution to a high-speed centrifuge and centrifuge at 10.000 × g for 30 sec at room temperature to remove the residual solution adsorbed on the wall of the centrifuge tube to maximize the recovery.
3. Add the mixture obtained in the previous step to the column G of the adsorption column provided by the kit (if it cannot be added at one time. 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. and the recovery rate of nucleic acid at a low concentration is significantly improved.
4. Add 600 µl Wash Buffer to the adsorption column G column. centrifuge at 13.000 × g for 1 min at room temperature. and discard the waste.
5. Repeat step 4
6. Centrifuge at 13.000 xg for 2 min at room temperature to thoroughly remove the residue of Wash Buffer.
7. 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 the residual ethanol. .
8. Add 15-50µl (recommended amount 30µl) to the middle of the adsorption column G column. Elution Buffer or ddH2O (the solution after 50°C water bath dissolves better). let the plasmid to be adsorbed completely dissolve for 2-5 min. 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)=OD260 × 50×Dilution factor.

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

Attention

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1. Before use. please check the Solution PG for crystallization or precipitation. If it is crystallized or precipitated. it can be clarified by a few minutes at 37 °C.
2. The recovery efficiency is related to the initial amount of DNA and the elution volume. The smaller the initial amount. the lower the elution volume and the lower the recovery rate.
3. All centrifugation steps can be carried out at room temperature.

Frequently Questions & Answers

Frequently Questions	Possible Causes	Suggestions
Low recovery or no banding	Too much PCR solution	The total volume of PCR fluid should be less than 300μl per time.
	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.
	Too few samples. too low concentration	Increase sample consumption
Recycled products cannot be used for subsequent experiments	Ethanol residue	When the room temperature is low. the drying time can be extended appropriately. Or put it in front of the air conditioner to dry the remaining ethanol.
	Salt residue	Make sure the amount of washing liquid and the number of washings are separated from each other by centrifugation.