

PCR product purification and recovery kit

Item No.: HDP208 100 times
HDP208M 200 times

Kit	composition	100time	200Time
Binding fluid GMB	Room temperature	100ml	200 ml
Rinsing liquid PE	Room temperature	50ml	100ml
Rinsing liquid WB	Room temperature	25 ml	50 ml
		Add the specified amount of ethanol according to the instructions before the first use	
Elution buffer EB	Room temperature	15 ml	15 ml
Adsorption column EC	Room temperature	100A	200A
Collection tube (2ml)	Room temperature	100A	200A

Product introduction:

This kit uses a unique binding solution system to purify and recover PCR products based on the combination of silicon matrix material and DNA. At the same time, it is removed by WB. Remove impurities such as protein, inorganic salt ions and oligonucleotide primers, purify DNA, and recover 100 bp-50 kb DNA fragments with a recovery rate of over 85%. The DNA recovered using this kit can be widely used in downstream experiments, including enzyme digestion, PCR, sequencing, ligation and transformation experiments.

Precautions

1. All centrifugation steps can be done at room temperature.
2. If the recovery rate is low, the pH value can be measured after the PCR product is added to the binding solution. After the sol, the sol solution still remains yellow and the pH is normal. If the pH value is greater than 7.5, the solution turns orange-red or lavender. Add 10-30 μ l of 3 M sodium acetate (pH 5.2) to adjust the pH value to between 5-7, and the solution is yellow.
3. When recovering DNA fragments of <100 bp and > 10 kb, the volume of the binding solution should be increased to extend the time of adsorption and elution.
4. The recovery rate is related to the initial DNA amount and elution volume. The smaller the initial amount and the elution volume, the lower the recovery rate.

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5. The amount of absolute ethanol in the rinsing solution WB before use, please tick in time after adding it to mark that ethanol has been added to avoid multiple additions!
6. When all the solutions have precipitation, they can be heated in a 37°C water bath to dissolve the precipitation. Return to room temperature before use.
7. After using each solution, close the lid in time.

Features

- a. Fast: The unique binding solution formula saves time and makes the experiment operation faster.
- b. Convenience: The experiment operation only needs simple steps such as rinsing and elution.
- c. Complete functions: ssDNA, dsDNA, and circular DNA can be recovered.
- d. High efficiency: the unique spin column and buffer ensure maximum efficiency in recovering high-purity target DNA.

DNA concentration and purity detection

DNA fragments can be tested for concentration and purity by agarose gel electrophoresis and UV spectrophotometer. DNA has an absorption peak at OD260, and an OD260 value of 1 is equivalent to about 50 µg/ml dsDNA and 40 µg/ml ssDNA. The OD260/OD280 ratio should be 1.7-1.9. If ddH₂O is used for elution, the ratio will be low, but it does not mean that the purity is low.

Steps:

Before using for the first time, add 4 times the volume of absolute ethanol to the rinsing solution WB!

1. The PCR product is directly added to the three-fold volume of the binding solution.
(Note: For recovery of small fragments <300 bp, 1.5 times the volume of isopropanol can be added at the same time to increase the recovery rate.)
2. Add the solution obtained in the previous step to an adsorption column EC, put the adsorption column in the collection tube, leave it at room temperature for 2 minutes, centrifuge at 12,000 rpm for 30-60 sec, discard the waste liquid in the collection tube, and place the adsorption column EC into the collection tube.

Note: The volume of the adsorption column is 750 µl. If the sample volume is larger than 750 µl, it can be added in batches.

Optional step: add 500µl of protein-removing solution PE, centrifuge at 12,000rpm for 30-60 seconds, and discard the waste solution.

3. Add 600 µl rinsing solution WB (please check whether absolute ethanol has been added before use!), centrifuge at 12,000 rpm for 1 min, discard the waste liquid, and put the adsorption column into the collection tube.
4. Repeat step 3.
5. Put the adsorption column EC back into the collection tube and centrifuge at 12,000 rpm for 2 minutes to remove the rinse solution. Put the adsorption column A at room temperature for a few minutes, and dry it thoroughly to prevent the residual rinsing solution from affecting the next experiment.

Note: The ethanol residue in the rinsing solution will affect the subsequent enzyme reaction (enzyme digestion, PCR, etc.) experiments.

6. Put the adsorption column EC into a clean centrifuge tube, and drop an appropriate amount of elution buffer EB into the middle of the adsorption membrane (the elution buffer can be preheated

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at 65-70 °C to increase the elution effect), and place at room temperature 2 min. Centrifuge at 12,000 rpm for 2 min to collect the DNA solution. In order to increase the amount of DNA recovered, the eluate obtained by centrifugation can be added back to the centrifugal adsorption column for elution, placed at room temperature for 2 min, centrifuged at 12,000 rpm for 2 min, and the DNA solution is collected in a centrifuge tube and stored at -20 °C.

Note: The elution volume should not be less than 30 μ l. Too little volume will affect the recovery efficiency. The pH value of the eluent should not be lower than 7.0. When subsequent experiments are used for sequencing, ddH₂O can be used as the eluent; DNA can also be eluted with 10 mM Tris-Cl, pH 8.0 buffer.

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