PCR Clean-up Kit / 96-well PCR Clean-up Kit

Kit contents, storage and stability

PCR Clean-up Kit

Cat. No	10-210310-05	10-210310-25
Preparation	50	250
DNA-prep Tube	50	250
2-ml Microfuge Tube	50	250
Buffer PCR-A	20 ml	100 ml
Buffer W1	28 ml	135 ml
Buffer W2	35 ml	88 ml╳2
Eluent	5 ml	25 ml
Handbook	1	1

96-well PCR Clean-up Kit

Cat. No	10-210310-04	10-210310-24
Preparation	4╳96	24╳96
96-well DNA-prep Plate	4	24
96-well Block (Square, 1.6 ml)	4	24
96-well V-shaped Bottom Plate	4	24
Buffer PCR-A	80 ml╳2	480 ml╳2
Buffer W1	50 ml	300 ml
Buffer W2	88 ml╳2	176 ml╳5
Eluent	25 ml	110 ml
Handbook	1	1

All buffers can be stored for at least two years without showing reduction in the efficiency of purification.

Buffer PCR-A: DNA binding buffer. Store at room temperature.
Buffer W1: Wash buffer. Store at room temperature.
Buffer W2: Desalting buffer. Before the use of the kit, add ethanol as much as indicated on the bottle and mix well. Store at room temperature.
Eluent: 2.5 mM Tris-HCl, pH8.5. Store at room temperature.

Introduction

IThe kit is suitable to purify DNA (longer than 100 bp) up to 8 g from reaction solutions after PCR, enzyme reaction, and sequencing. The typical yield is 70~90%. It is not necessary to remove mineral oil. The purified DNA is free from primers less than 50 mer, enzyme proteins, mononucleotide, and mononucleotide labelled with fluorescent dye or radioactive isotope, and can be applied in biological experiments such as sequencing, digestion, ligation, in vitro transcription, microinjection and gene chip-based analysis etc.

Principle

Buffer PCR-A contains high concentration of chaotropic salt, which allows DNA longer than 100 bp in length to bind selectively to silica membrane. The primers less than 50 mer, enzyme proteins, mononucleotide, and mononucleotide labelled with fluorescent dye or radioactive isotope are passed through column and removed. After washing with Buffer W1 and Buffer W2, the DNA binding on membrane can be eluted by water or eluent, and can be applied in all sorts of molecular biological experiments.

Preparation before experiment

1) Before the use of the kit, add ethanol as much as indicated on the bottle and mix.
2) Check Buffer PCR-A for precipitation before each use. If precipitation occurs, incubate at 65℃ to dissolve the precipitate.
3) Pre-warming water or Eluent at 65℃ will improve elution efficiency.

Notes

Buffer PCR-A and Buffer W1 contain irritant compound. When working with the buffers, always wear suitable protective clothing such as laboratory overalls, safety glasses and gloves. Take care to avoid contact with skin or eyes. In the case of contact with skin or eyes, wash immediately with water. If necessary, ask for medical assistance.

Protocol

I. PCR Clean-up by spin column

1. Add 3-sample volume of Buffer PCR-A into sample. If the required volume of Buffer
PCR-A is less than 100 l, add 100 l of Buffer PCR-A.

Either Vacuum Filtration with Vacuum Manifold or Centrifugation can be chosen to proceed Step2~4.

A. Vacuum Filtration

2A. Attach the Vacuum Manifold Base to a vacuum pump. Transfer the binding mix from Step1 to the DNA-prep Tube. Switch on vacuum source and adjust vacuum to draw solution slowly through the DNA-prep Tube (Keep flow rate at about one drop per second).
3A. Turn vacuum to the maximum, add 500 l of Buffer W1 and drain off the solution through the tube.
4A. Keep vacuum. Add 700 l of Buffer W2 along the wall of DNA-prep Tube to wash off Buffer W1 on the wall, and draw through the tube. Wash once again with 700 l of Buffer W2.

* Make sure that ethanol has been added into Buffer W2 .
* Add Buffer W2 along the tube wall to wash off the salt binding on it.

B. Centrifugation

2B. Place DNA-prep Tube in the 2-ml Microfuge Tube. Transfer the binding mix from Step 1 to the DNA-prep Tube. Centrifuge at 2500×g (or 5500 rpm) for 1 min.
3B. Discard the flow-through in the 2-ml Microfuge Tube. Place the DNA-prep Tube back to the 2-ml Microfuge Tube. Add 500 l of Buffer W1 to the DNA-prep Tube and centrifuge at 2500×g (or 5500 rpm) for 1min.
4B. Discard the flow-through and place the DNA-prep Tube back to the 2-ml Microfuge
Tube. Add 700 l of Buffer W2, and centrifuge at 2500×g (or 5500 rpm) for 1min. Discard the flow-through and wash once again with 700 l of Buffer W2.

* Make sure that ethanol has been added into Buffer W2 .

5. Transfer the DNA-prep Tube in a 1.5-ml microfuge tube and centrifuge at 12000×g

for 1 min.
6. Transfer the DNA-prep Tube in a clean 1.5-ml microfuge tube. To elute DNA, add 25~30 l of water or the Eluent to the centre of the membrane, let it stand for 1 min at room temperature. Centrifuge at 12000×g for 1 min.

II. 96-well PCR Clean-up

A. Preparation by using Vacuum Filtration

1A. Add 3-sample volume of Buffer PCR-A into samples. If the required volume of Buffer PCR-A is less than 100 l, add 100 l of Buffer PCR-A.
2A. Attach the Vacuum Manifold with a vacuum pump. Assemble Vacuum Manifold. Place 96-well DNA-prep Plate onto Vacuum Manifold. Transfer the binding mix from Step 1A to the corresponding well of the Plate. Switch on vacuum source and adjust vacuum to draw solution slowly through the 96-well DNA-prep Plate.
3A. Turn vacuum to the maximum, add 100 l of Buffer W1 and drain off the solution through the 96-well DNA-prep Plate.
4A. Keep vacuum. Add 350 l of Buffer W2 along each well of the 96-well DNA-prep Plate, and drain off all the solution in the 96-well DNA-prep Plate.

* Make sure that ethanol has been added into Buffer W2.

5A. Switch off the Vacuum Manifold, and release vacuum inside. Tap the Plate 6 times on paper towel to drain off completely the solution remaining on the bottom.
6A. Place the Plate back onto the Vacuum Manifold, and Turn vacuum to the maximum. Add 350 l of Buffer W2 along each well of the Plate, and drain off all the solution in the Plate.
7A. Place the Plate onto 96-well Block (square, 1.6ml), and centrifuge for 5 min at 3000×g.
8A. Transfer the Plate onto 96-well V-shaped Bottom Plate. Add 25~30 l Eluent or water to the centre of membrane, and let it stand at room temperature for 1 min. Centrifuge for 2 min at 3000×g to elute DNA.

B.Preparation by Centrifugation

1B. Add 3-sample volume of Buffer PCR-A into samples. If the required volume of Buffer PCR-A is less than 100 l, add 100 l of Buffer PCR-A.
2B. Place the 96-well DNA-prep Plate onto 96-well Block (square, 1.6 ml), transfer the binding mix from Step 1B to the corresponding well of the 96-well DNA-prep Plate. Centrifuge for 1 min at 1000×g.

* It is not necessary to discard the flow-through in the Block.
3B. Add 100 l of Buffer W1 along each well of the 96-well DNA-prep Plate. Centrifuge

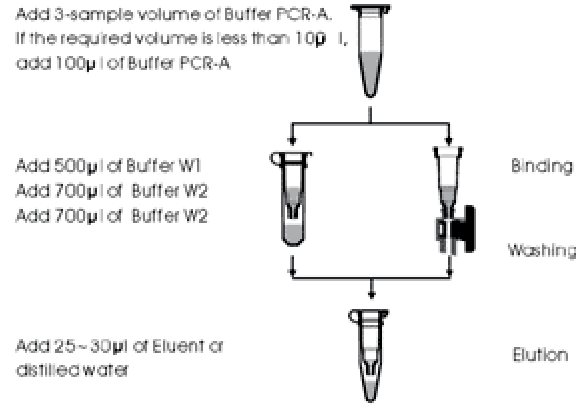
for 1 min at 1000×g.

* It is not necessary to discard the flow-through in the Block.

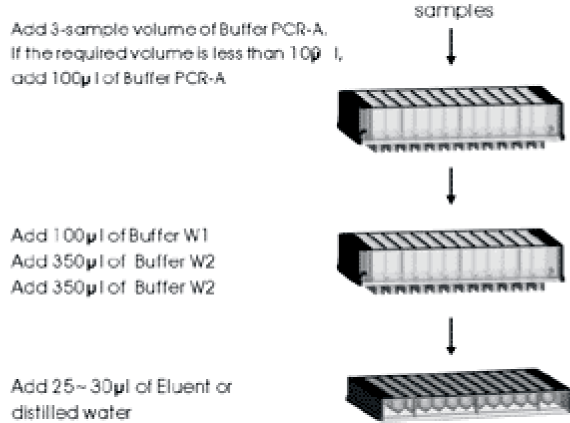
4B. Add 350 l of Buffer W2 along each well of the 96-well DNA-prep Plate. Centrifuge for 1 min at 1000×g.
5B. Discard the flow-through in the Block. Place the 96-well DNA-prep Plate back onto
96-well Block (square, 1.6 ml). Add 350 l of Buffer W2. Centrifuge for 5 min at 3000×g.
6B. Transfer the 96-well DNA-prep Plate onto 96-well V-shaped Bottom Plate. Add 25~30 l of Eluent or water to the centre of membrane, and let it stand at room temperature for 1 min. Centrifuge for 2 min at 3000×g to elute DNA.

Operation procedure

I. PCR Clean-up by spin column



II. 96-well PCR Clean-up



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