Molecular cloning and genetic engineering – PCR Purification

Aim

This kit is suitable for extracting up to 8 μg DNA (more than 75bp) from the reaction solution of PCR, enzymatic reaction and sequencing reaction. The recovery rate is 70-90%. The purified DNA doesn’ t contain primers, enzyme-proteins and single nucleotides.

Materials

Cat. No.	AP-PCR-4	AP-PCR-50	AP-PCR-250
Preparation times	4 Preps	50 Preps	250 Preps
PCR preparation tube	4	50	250
1.5 mL centrifuge tube	4	50	250
2 mL centrifuge tube	4	50	250
Buffer PCR-A	2 mL	20 mL	100 mL
Buffer W2 concentrate	2.4 mL	24 mL	2* 72 mL
Eluent	1 mL	5 mL	25 mL
Instruction	1	1	1

Buffer PCR-A: DNA binding solution. Stored in airtight condition at room temperature.

Buffer W2 concentrate: Desalination solution. Before use, absolute ethanol (100% ethanol or 95% absolute ethanol) is added according to the specified

volume on the reagent bottle and then well mixed, stored in airtight condition at room temperature.

Eluent: 2.5 mM Tris-HCl, pH 8.5, stored in airtight condition at room temperature.

Procedure

1. Preparation for Experiments

(1) Prepare Tip head and centrifuge tubes which are nucleic acid and nuclease free.

(2) Before the first use, adding absolute ethanol to Buffer W2 concentrate according to the volume specified on the reagent bottle.

(3) Before use, check whether there is precipitation in Buffer PCR-A. If there is precipitation, it should be heated in 65 ℃ warm water-bath until the precipitation is completely dissolved and cooled down to room temperature before use.

(4) Heating Eluent or deionized water to 65 ℃ is beneficial to improve the elution efficiency enzymatic reactions. They are right about this.

2. Operation Steps

Users can choose negative pressure method or centrifugal method.

Negative pressure method

1A. Connect the negative pressure device correctly and insert the preparation tube into the outlet of the device; add three volumes of Buffer PCR-A (if the Buffer PCR-A is less than 100 ml, add to 100 ml) into the reaction solution of PCR, endonuclease, enzyme labeling or sequencing. Mix it and transfer it to the preparation tube. Open and adjust the negative pressure to -25-30 inch/Hg, and slowly remove the solution from the tube.

2A. Add 700 μL Buffer W2 and remove the solution in the tube. Wash again with 700 μL Buffer W2 in the same way.

* Make sure that absolute ethanol has been added to the Buffer W2 concentrate according to the specified volume of the reagent bottle.

* Washing with Buffer W2 two times ensures that the salt is completely removed, eliminating the effect on the endonuclease reaction.

3A. The preparation tube was placed in a 2 ml centrifuge tube (provided in the kit) and centrifuged for 1 minute at 12,000 x g.

4A. Place the preparation tube in a clean 1.5 ml centrifuge tube (provided in the kit), add 25-30 ml Eluent or deionized water in the center of the preparation tube membrane, and leave it at room temperature for 1 minute. DNA was eluted by centrifugation for 1 minute at 12,000 x g.

* Heating Eluent or deionized water to 65 ℃ will improve the elution efficiency.

Centrifugal method

1B. Add three volumes of Buffer PCR-A (if Buffer PCR-A is less than 100 μL, add it to 100 μL) in the reaction solution of PCR, endonuclease, enzyme labeling or sequencing. Mix it and transfer it to the preparation tube. Place the preparation tube in a 2 ml centrifuge tube (provided in the kit), centrifuge for 1 minute at 12,000 x g, and discard the filtrate.

2B. Put the preparation tube back into a 2 ml centrifuge tube, add 700 ml Buffer W2, centrifuge for 1 min at 12,000 x g, discard the filtrate.

* Make sure that absolute ethanol has been added to the Buffer W2 concentrate according to the specified volume of the reagent bottle.

3B. (Optional) Place the preparation tube in the centrifuge tube, then place the preparation tube back to a 2 ml centrifuge tube, add 400 ml Buffer W2, centrifuge for 1 minute at 12,000 x g.

* It is suggested to remind customers that when removing 2 ml centrifugal tube from the centrifuge. Note: Do not let Buffer W2 at the bottom of the tube contact with the preparation tube.

4B. Place the preparation tube in a clean 1.5 ml centrifugal tube (provided in the kit). Add 25-30 ml Eluent or deionized water in the center of the preparation tube and place it at room temperature for 1 minute. DNA was eluted by centrifugation for 1 minute at 12,000 x g.

* Heating Eluent or deionized water to 65 ℃ will improve the elution efficiency.

Note

1. Buffer PCR-A contains irritant compounds. Wear latex gloves and glasses when operating. Avoid contact with skin, eyes and clothes. Beware of inhalation of mouth and nose. If skin or eyes are contaminated, rinse them immediately with plenty of water or saline, and seek medical advice if necessary.

2. The DNA molecule is acidic and is recommended to be stored in 2.5 mM Tris-HCl and pH 8.5 eluent.