

DNA Clean-up Kit

Cat. No. : CW2301S (50 preps)
CW2301M (200 preps)

Storage Condition: Store at room temperature (15-30°C).

Components

Component	CW2301S (50 preps)	CW2301M (200 preps)
Buffer PB	30 mL	120 mL
Buffer PS	15 mL	60 mL
Buffer PW (concentrate)	6 mL	25 mL
Buffer EB	10 mL	30 mL
Spin Columns DM with Collection Tubes	50	200

Introduction

The kit uses new silicon matrix membrane technology and reagent formula, and can purify and recover 100 bp–10 kb DNA fragments from PCR products or enzyme reaction solution (enzyme digestion, ligation, probe labeling, etc.) through three steps of fast and simple binding – washing – elution. Each adsorption column can absorb up to 10 µg of DNA and maximize the removal of primers, oligonucleotides, enzymes and other impurities. The purified DNA has high purity and concentration, good integrity and high recovery, and can be directly used in molecular biology experiments such as sequencing, ligation and transformation, labeling, in vitro transcription.

Reagents to be Supplied by user

100% ethanol

Precautions

1. All components can be stored stably in dry, room temperature (15–30°C) for 1 year, and stored at 2–8°C for a longer time. When the solution is preserved at low temperature, it needs to be restored to room temperature.
2. This kit can unselectively recover all DNA fragments in the solution. If you need to selectively recover specific fragments and remove other fragments of different sizes, please choose our Gel Extraction Kit (Cat. No.: CW2302).
3. 100% ethanol should be added to the Buffer PW according to the instructions on the reagent bottle label before the first use.
4. Please check whether there is crystallization or precipitation in Buffer PB before use. If there is crystallization or precipitation, you can take a water bath at 37°C for a few minutes to restore clarification.
5. Recovery efficiency is related to the initial DNA amount and elution volume. The smaller the initial amount, the smaller the elution volume, and the lower the recovery rate.
6. All centrifugation steps can be carried out at room temperature.

Protocol

1. Estimate the volume of the DNA reaction solution, add 5 times the volume of Buffer PB, and mix well (no need to remove paraffin oil or mineral oil).
Note: 1) If the DNA reaction system is 50 µL (excluding paraffin oil volume), add 250 µL Buffer PB. 2) Check the PH value of the solution after adding Buffer PB. If the PH >7.5, add 10–30 µL 3M Sodium acetate (pH=5.0) to adjust pH to 5–7.
2. Column balance: Add 200 µL Buffer PS to the Spin Columns DM with Collection Tube, centrifuge at 13,000 rpm (~16,200 ×g) for 1 min. Discard the waste liquid, and put the adsorption column back into the collection tube.
3. Add the solution obtained in step 1 to the Spin Columns DM with Collection Tube, leave it at room temperature for 1 min, centrifuge at 13,000 rpm for 30–60 s. Discard the waste liquid, and put the adsorption column back into the collection tube.
Note: The adsorption column volume is 750 µL. If the sample volume is more than 750 µL, it can be added in batches.
4. Add 500 µL Buffer PW (please check whether 100% ethanol has been added before use) to the adsorption column, centrifuge at 13,000 rpm for 30–60 s. Discard the waste liquid, and put the adsorption column back into the collection tube.
Note: If the purified DNA is used for salt sensitivity tests (such as blunt-end ligation test or direct sequencing), it is recommended to add Buffer PW and leave it at rest for 2–5 min before centrifugation.
5. Centrifuge at 13,000 rpm for 1 min and discard the waste liquid in the collection tube. Leave the column at room temperature for several minutes to dry thoroughly.
Note: The purpose of this step is to remove the residual ethanol in the adsorption column. The residual ethanol will affect subsequent enzymatic reactions (enzyme digestion, PCR, etc.). To ensure that downstream experiments are not affected by residual ethanol, it is recommended to open the adsorption column and leave it at room temperature for a few minutes to completely dry out the residual ethanol in the adsorption material.
6. Put the adsorption column into a new centrifuge tube (self-provided), add 30–50 µL Buffer EB to the middle of the adsorption membrane, leave it at room temperature for 1 min, 13,000 rpm centrifuge for 1 min to collect the DNA solution. Store DNA at –20°C
Note: 1) The pH of the elution buffer has a great influence on the elution efficiency. If water is used as elution buffer, the pH should be between 7.0 and 8.5 (use NaOH to adjust) . 2) In order to improve the recovery rate of DNA, the solution obtained by centrifugation can be added back to the adsorption column, left at room temperature for 2 min, and centrifuged at 13,000 rpm for 1 min. 3) The elution volume should not be less than 30 µL. Too little volume will affect recovery efficiency. 4) When DNA fragments are larger than 10 kb, Buffer EB should be preheated in a 50°C water bath to increase recovery efficiency.