CYDR09-CHANGYU PCR Purification Midi Kit

Catalog number	Package
CYDR0901	50 preps

Storage and shipping: room temperature only; do not store at 4 °C or -20 °C, because precipitation reduces performance. Stable for 12 months at room temperature. All solutions should be clear; if a precipitate forms at low ambient temperature, do not use the solution directly - warm it in a 37 °C water bath for a few minutes until clear, then bring it to room temperature before use. All solutions should be kept tightly capped after use to avoid evaporation, oxidation and pH drift.

Description

In the presence of a high concentration of chaotropic salt, DNA fragments selectively bind to the silica membrane of the spin column. A series of rapid wash and centrifugation steps remove primers, nucleotides, proteins, enzymes and other impurities. Purified DNA is then eluted from the silica membrane with a low-salt, high-pH elution buffer. The thickened adsorbent membrane binds about 2.5 times more DNA than conventional PCR product cleanup kits and can bind up to 20-30 μg of DNA, making the kit suitable for medium- to large-scale PCR product purification.

Kit components

Component	Storage	50 preps (CYDR0901)
Equilibration Buffer	RT	5 ml
Binding Buffer BB	RT	50 ml
Wash Buffer WB	RT	13 ml (add ethanol before first use)
Elution Buffer EB	RT	10 ml
Spin column TC (thickened)	RT	50
Collection tubes (2 ml)	RT	50

Store the kit at room temperature. All solutions should be kept tightly capped after use to avoid evaporation, oxidation and pH drift.

Features

The silica membrane of each spin column is a specially manufactured adsorbent membrane; binding varies little between columns and reproducibility is good.

The binding buffer is of high quality and contains neither sodium iodide nor perchlorate, so it does not inhibit downstream digestion, ligation or cloning.

Binding Buffer BB contains phenol red, giving a yellow colour that helps to monitor pH changes for optimal binding and improves recovery efficiency.

The improved binding buffer formulation has high buffering capacity and stability; the pH stays in the optimal binding range even when samples vary, without adding acetic acid.

The procedure is rapid and convenient; no toxic phenol or chloroform and no ethanol precipitation are required.

Notes before starting

Note: All centrifugation steps are carried out at room temperature in a standard benchtop centrifuge at up to 12,000 rpm.

Note: The binding buffer and equilibration buffer contain irritants. Wear gloves and avoid contact with skin and eyes. If contact occurs, rinse immediately with plenty of water or saline.

Note: Recovery is generally intended for DNA fragments between 70 bp and 40 kb; recovery efficiency decreases rapidly for fragments that are too long or too short. The kit also recovers small fragments such as 70 bp efficiently.

Note: The amount of recovered DNA depends on the amount of starting DNA, the elution volume and the DNA fragment size. For 5-25 μg of 100 bp-5 kb DNA, recovery can be as high as 95%.

Note: Elution Buffer EB contains no EDTA and does not affect downstream digestion or ligation. Water may also be used for elution if its pH is above 7.5; a low pH reduces elution efficiency. DNA eluted with water should be stored at -20 °C. For long-term storage, DNA may be eluted with TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0); EDTA may affect downstream digestion and the TE eluate can be diluted if necessary.

❖ Procedure

Before first use, add the volume of absolute ethanol indicated on the label to Wash Buffer WB and tick the box on the label.

1. Column equilibration: apply 100 μl of equilibration buffer to spin column TC, centrifuge at 13,000 rpm for 1 minute, discard the flow-through and set the column aside. Equilibration buffer enhances nucleic acid binding; use columns equilibrated on the same day.

2. Add Binding Buffer BB to the PCR amplification product or restriction digestion product at a ratio of 5:1 (binding buffer : sample) and mix thoroughly (for example, add 500 μl of Binding Buffer BB to 100 μl of PCR or digestion product and mix well).

3. Load the solution from the previous step onto spin column TC placed in a collection tube. Leave at room temperature for 1 minute, centrifuge at 13,000 rpm for 1 minute and discard the flow-through. If the total volume exceeds 750 μl, load it in two portions onto the same column.

If the flow-through mixes with residual strongly alkaline equilibration buffer in the collection tube, the binding buffer may change from yellow to orange-red or purple. This is the normal colour change of the phenol red pH indicator under alkaline conditions.

4. Add 600 μl of Wash Buffer WB (check that absolute ethanol has been added), centrifuge at 13,000 rpm for 30 seconds and discard the flow-through. Repeat the wash once with another 600 μl of Wash Buffer WB and discard the flow-through.

5. Return the column to the empty collection tube and centrifuge at 13,000 rpm for 2 minutes to remove residual wash buffer, since residual ethanol can inhibit downstream reactions.

6. Transfer the column to a clean centrifuge tube. Apply 50-100 μl of Elution Buffer EB to the centre of the membrane (prewarming EB to 80-90 °C increases yield), leave at room temperature for 2 minutes and centrifuge at 13,000 rpm for 1 minute; discard the column.

To increase DNA recovery, reload the eluate onto the column, leave for 1 minute and centrifuge again; a second elution increases the concentration by about 10%. A larger elution volume increases recovery; if a higher DNA concentration is needed, reduce the elution volume, but not below 50 μl, since too small a volume reduces DNA recovery and yield.

This product is furnished for LABORATORY RESEARCH USE ONLY. Not for diagnostic or therapeutic use.