CYDR03-CHANGYU Universal Gel/DNA Purification Kit

Catalog number	Package
CYDR0301	50 preps
CYDR0302	100 preps
CYDR0303	200 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.

Kit components

Component	Storage	50 preps (CYDR0301)	100preps (CYDR0302)	200preps (CYDR0303)
Equilibration Buffer	RT	5 ml	10 ml	20 ml
Solubilization/Binding Buffer HB (high concentration)	RT	20 ml	40 ml	80 ml
Wash Buffer WB	RT	13 ml	25 ml	50 ml (add ethanol before first use)
Elution Buffer EB	RT	10 ml	10 ml	15 ml
Spin column EC	RT	50	100	200
Collection tubes (2 ml)	RT	50	100	200

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

The high-concentration Solubilization/Binding Buffer HB allows solubilization at as little as one volume, reducing handling steps.

Solubilization and binding are combined in one buffer, so a single kit can be used for agarose gel DNA recovery, PCR product cleanup and restriction fragment purification.

Solubilization/Binding Buffer HB contains phenol red, giving a yellow colour that helps to monitor solubilization and pH changes for optimal binding.

The improved HB 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: Solubilization/Binding Buffer HB 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 fragment size. For 1-15 µg of 100 bp-5 kb DNA, recovery can be as high as 85%.

Note: UV observation damages DNA fragments during gel excision; use low-energy long-wave UV and keep exposure as short as possible.

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.

A. Agarose gel DNA recovery

1. Under long-wave UV light, cut out the required DNA band with a clean blade. Remove as much gel as possible that does not contain DNA; the smaller the gel volume, the better.

2. Cut the gel slice containing the DNA band into small pieces, place them in a 1.5 ml centrifuge tube and weigh. Weigh an empty 1.5 ml tube first, then weigh again with the gel; the difference is the gel weight.

3. Add 100 µl of Solubilization/Binding Buffer HB (high concentration) per 100 mg of 1% agarose gel. For gels of higher agarose concentration, increase the volume of Buffer HB proportionally. To simplify the procedure, a fixed volume of 400 µl of Buffer HB may be used.

4. Incubate in a 56 °C water bath for 10 minutes, or until the gel is completely dissolved. Vortex every 2-3 minutes to speed up dissolution.

5. Optional and generally not required: for fragments below 400 bp, add 0.3 volume of isopropanol to improve recovery.

6. Column equilibration: apply 100 µl of equilibration buffer to spin column EC, centrifuge at 12,000 rpm for 1 minute, discard the flow-through and set the column aside. Equilibration buffer enhances nucleic acid

binding to the silica membrane; use columns equilibrated on the same day.

7. Load the solution from the previous step onto spin column EC placed in a collection tube. Leave at room temperature for 1 minute, centrifuge at 12,000 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, Buffer HB may change from yellow to orange-red or purple. This is the normal colour change of the phenol red pH indicator under alkaline conditions.

8. Add 600 µl of Wash Buffer WB (check that absolute ethanol has been added), centrifuge at 12,000 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.

9. Return the column to the empty collection tube and centrifuge at 12,000 rpm for 2 minutes to remove residual wash buffer, since residual ethanol can inhibit downstream reactions.

10. Transfer the column to a clean centrifuge tube. Apply 50 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 12,000 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 25 µl, since too small a volume reduces DNA recovery and yield.

B. Purification of PCR products or restriction fragments

1. Estimate the volume of the PCR or restriction digestion reaction and add 3 volumes of Solubilization/Binding Buffer HB (high concentration). Mix thoroughly; removal of paraffin oil or mineral oil is not required.

2. Column equilibration: apply 100 µl of equilibration buffer to spin column EC, centrifuge at 12,000 rpm for 1 minute, discard the flow-through and set the column aside.

3. Load the solution onto spin column EC placed in a collection tube. Leave at room temperature for 1 minute, centrifuge at 12,000 rpm for 1 minute and discard the flow-through.

4. From this step on, follow steps 8-10 of Section A exactly.

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