# CYDR07-CHANGYU miRNA PAGE Extraction Kit

| Catalog number | Package |
|---|---|
| CYDR0701 | 50 preps |

Storage and shipping: room temperature. Stable for 12 months at room temperature. All solutions should be kept tightly capped after use to avoid evaporation, oxidation and pH drift.

## Description

The kit uses a novel silica-membrane spin column and a dedicated buffer system to recover small RNA fragments of 200 nt or less from polyacrylamide gels simply and efficiently, especially microRNA (miRNA) molecules of about 20 nt, with recovery up to 85%. Impurities are removed to the greatest extent possible, giving highly pure RNA that can be used directly for reverse transcription, miRNA sequencing library construction and other downstream molecular biology experiments.

## Kit components

| Component | Storage | 50 preps (CYDR0701) |
|---|---|---|
| Equilibration Buffer | RT | 5 ml |
| Binding Buffer MRB | RT | 40 ml (add isopropanol before first use) |
| Buffer DB | RT | 30 ml |
| Wash Buffer RW | RT | 10 ml (add ethanol before first use) |
| RNase-free H2O | RT | 10 ml |
| Spin column EC | 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

RNA of various lengths in the range of 15-3000 nt can be recovered, including miRNA fragments below 100 nt.

The binding buffer is of high quality and contains neither sodium iodide nor perchlorate, so it does not inhibit downstream reverse transcription or sequencing library construction.

The formulation gives high recovery efficiency.

The spin-column format 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 13,000 rpm.

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

**Note:** Use fresh electrophoresis running buffer to avoid affecting electrophoresis and recovery.

**Note:** Keep UV exposure as short as possible during gel excision to avoid RNA damage. Alternatively, stain with a visible-light dye and excise the gel under visible light to avoid UV damage.

**Note:** Recovery depends on fragment size, gel concentration, the amount of input RNA and the elution volume.

### Using the equilibration buffer

Silica-membrane spin columns can lose nucleic acid binding capacity during long storage through reaction with charges and dust in the air. Pretreatment with the equilibration buffer greatly reduces the hydrophobic groups of the silica membrane and improves nucleic acid binding, increasing recovery or yield. The equilibration buffer is strongly alkaline; if it contacts the skin, rinse with plenty of tap water. Keep the bottle tightly capped after use and store at room temperature. Precipitate may form during storage; heat to 37 °C until it dissolves completely.

Place a new spin column in a collection tube, apply 100 µl of equilibration buffer to the column, centrifuge at 13,000 rpm for 1 minute, discard the flow-through and return the column to the collection tube. The column is now equilibrated; continue with the procedure.

## ❖ Procedure

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

1. Excise the PAGE gel containing the RNA fragment (about 100 mg), removing as much excess gel as possible since it reduces recovery. Place the gel in a 1.5 ml centrifuge tube and crush it as finely as possible with a pipette tip (ideally seal the tip opening over an alcohol flame first).

2. Add 1-2 volumes of Buffer DB to the gel (for example, 100-200 µl of Buffer DB per 100 mg of gel) and incubate at 55 °C for 30 minutes to 2 hours, vortexing every 15 minutes to promote diffusion of RNA into the solution.

In general, larger fragments require longer diffusion times. For RNA fragments of about 100 nt or less, about 30 minutes is sufficient (longer incubation does not reduce recovery); for larger RNA fragments, the incubation may be extended appropriately.

3. Centrifuge at 13,000 rpm for 5 minutes. Carefully transfer the supernatant to a new centrifuge tube and record the supernatant volume.

4. Add 9 volumes of Binding Buffer MRB to the supernatant and mix. For fragments larger than 200 nt, 5 volumes of Binding Buffer MRB are sufficient.

5. Equilibrate spin column EC with equilibration buffer. This is a mandatory step; see 'Using the equilibration buffer' above.

6. Load the solution from the previous step onto spin column EC placed in a collection tube. Leave at room temperature for 1 minute and centrifuge at 13,000 rpm for 30 seconds; discard the flow-through. The maximum column volume is 750 µl; if the solution volume is larger, load it in portions.

7. Add 500 µl of Wash Buffer RW (check that absolute ethanol has been added), centrifuge at 13,000 rpm for 30 seconds and discard the flow-through. Repeat the wash once.

8. Return column EC 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.

9. Transfer column EC to a clean centrifuge tube and apply 30-50 µl of RNase-free H2O to the centre of the membrane. Leave at room temperature for 2 minutes and centrifuge at 13,000 rpm for 1 minute. If a higher nucleic acid concentration is required, reload the eluate onto the column and centrifuge for 1 minute.

A larger elution volume increases recovery; if a higher concentration is needed, reduce the elution volume, but not below 25 μl, since too small a volume reduces nucleic acid recovery and yield.

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