# CYRN72-CHANGYU RNAclean RNA Clean Micro Kit

| Catalog number | Package |
|---|---|
| CYRN7201 | 50 preps |

Storage and shipping: room temperature. Stable for 12 months. Keep all solutions tightly capped after use.

## Description

This kit uses spin columns fitted with a specially selected silica membrane; batch-to-batch variation in binding capacity is minimal and reproducibility is high. RNA binds efficiently and specifically to the membrane under high-salt conditions while proteins, inorganic ions and many organic impurities are removed; RNA is eluted under low-salt conditions. The specially designed ultra-micro column allows recovery of micro- to ultra-micro-scale samples, typically 0.1-10 μg of RNA. The kit is used to purify and recover RNA from enzymatic reactions (for example DNase treatment, protease treatment or RNA labeling) and to purify RNA obtained by other extraction methods. The purified total RNA is free of protein contamination and is suitable for Northern blot, dot blot, mRNA isolation, cDNA synthesis, primer extension and differential display.

## Kit components

| Component | 50 preps (CYRN7201) |
|---|---|
| Binding Buffer RC | 20 ml |
| Wash Buffer RW | 10 ml (add ethanol before first use) |
| RNase-free H2O | 5 ml |
| RNase-free ultra-micro spin columns | 50 |
| Collection tubes (2 ml) | 50 |

## Features

A gasket-free column design ensures no residual liquid or contamination after centrifugation, giving highly pure recovered RNA.

The ultra-micro column has a much smaller membrane area, minimizing loss of RNA adsorbed to the membrane during recovery, and allows elution in as little as 6 μl, giving high RNA concentration.

Unlike other suppliers' ultra-micro columns, this column uses a hydrophobic, nucleic-acid-non-binding support under the silica membrane that withstands centrifugation up to 20,000 rpm without the membrane detaching, ensuring that impurities, salts and ethanol are removed as completely as possible.

## ❖ Procedure

Before first use, add absolute ethanol to Wash Buffer RW as indicated on the label and tick the box to avoid adding it twice. Perform all steps at room temperature and work quickly to minimize RNA degradation.

1. On ice, adjust the RNA sample to 100 μl with RNase-free water and add 350 μl of Binding Buffer RC. Mix well.

2. Add 250 μl of absolute ethanol and mix. No centrifugation is required.

3. Transfer the solution, together with any precipitate, to an ultra-micro column placed in a collection tube. Centrifuge at room temperature (all subsequent steps are at room temperature) at 13,000 rpm for 30 seconds, discard the flow-through and return the column to the collection tube.

If residual DNA must be removed, on-column DNase digestion can be performed after this step; see the Appendix.

4. Add 500 µl of Wash Buffer RW (check that ethanol has been added), centrifuge at 13,000 rpm for 15 seconds and discard the flow-through.

5. Repeat step 4 once.

6. Return the ultra-micro 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.

7. Transfer the ultra-micro column to a new 1.5 ml centrifuge tube. Apply 6-15 µl of RNase-free ddH2O to the centre of the membrane (prewarming to 90-100 °C increases the recovered RNA concentration), leave for 2 minutes and centrifuge at 13,000 rpm for 1 minute.

Use an elution volume of at least 5 µl; smaller volumes reduce recovery. Reloading the first eluate onto the column for a second elution increases the concentration by about 10%.

## ❖ Appendix: On-column DNase I digestion

This kit can also be used for on-column DNase digestion to remove trace DNA contamination. For stringent mRNA expression analysis such as real-time PCR, the DNase I on-column digestion kit (CYRN34, catalog number CYRN3401) can be used to digest DNA directly on the membrane of the ultra-micro column, after which the purified RNA is eluted and used directly. Deproteinization Buffer RW1 can be purchased separately if needed.

Example using the CYRN34 DNase I on-column digestion kit: mix 20 µl of DNase I buffer and 2 µl of RNase-free DNase I gently to prepare the working solution (scale up proportionally for multiple columns). If residual DNA is high and digestion is incomplete, increase the amount of enzyme proportionally (for example 50 µl of DNase I buffer and 5 µl of RNase-free DNase I).

1. Perform the normal procedure up to the end of step 3, then continue as follows.

2. Add 350 µl of Deproteinization Buffer RW1 to the ultra-micro column, centrifuge at 12,000 rpm for 30 seconds, discard the flow-through and return the column to the collection tube.

3. Apply 22 µl of DNase I working solution to the centre of the membrane and leave at room temperature (20-30 °C) for 15 minutes. Apply the solution directly to the membrane; do not let it run onto the O-ring or the column wall.

4. Add 350 µl of Deproteinization Buffer RW1 to the ultra-micro column, centrifuge at 12,000 rpm for 30-60 seconds, discard the flow-through and return the column to the collection tube.

5. Continue with the Wash Buffer RW steps and the remaining steps of the procedure. When using another supplier’s kit, continue with its final wash step.

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