CYRN14-CHANGYU RNAclean RNA Clean Kit

Catalog number	Package
CYRN1401	50 preps

Storage and shipping: room temperature. Stable for 12 months. Keep all solutions tightly capped after use to avoid evaporation, oxidation and pH drift.

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. Up to 50 µg of RNA can be processed per column. 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 (CYRN1401)
Binding Buffer RC	20 ml
Wash Buffer RW	10 ml (add ethanol before first use)
RNase-free H2O	5 ml
RNase-free spin columns RA	50
Collection tubes (2 ml)	50

Before first use, add absolute ethanol to the Wash Buffer RW bottle as indicated on the label and tick the box to confirm that ethanol has been added.

Notes before starting

Note: All centrifugation steps are carried out at room temperature at 13,000 rpm in a conventional benchtop centrifuge.

Note: For very small amounts of RNA (for example a few hundred nanograms), the CYRN72 ultra-micro RNA cleanup kit (catalog number CYRN7201) is recommended to recover micro-scale samples.

❖ Procedure

Add absolute ethanol to Wash Buffer RW before first use as indicated on the label and tick the box to avoid adding ethanol 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 from the previous step, together with any precipitate, to spin column RA 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 column RA 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 column RA to a new 1.5 ml centrifuge tube. Apply 50-80 μl of RNase-free ddH2O to the centre of the membrane, leave for 2 minutes and centrifuge at 13,000 rpm for 1 minute.

Use an elution volume of at least 30 μl; smaller volumes reduce recovery. To increase RNA concentration, prewarm the RNase-free ddH2O to 90-100 °C before elution, leave it on the membrane for 2-3 minutes at room temperature, or reload the first eluate onto the column for a second elution.

❖ 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 column RA, 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: prepare the DNase I working solution by mixing 45 μl of DNase I buffer and 5 μl of RNase-free DNase I gently (scale up proportionally for multiple columns). If residual DNA is high and digestion is incomplete, the amount of enzyme can be increased proportionally (for example 90 μl of DNase I buffer and 10 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 column RA, centrifuge at 13,000 rpm for 30 seconds, discard the flow-through and return the column to the collection tube.

3. Apply 50 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 column RA, centrifuge at 13,000 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.