CYRN03-RNApure Total RNA Kit

Cat#：CYRN03

Kit Contents	Storage	50 Preps (CYRN0302)
Buffer RL (TRIzol® Reagent)	4 °C	50 ml
Wash Buffer RE	RT	25 ml
Wash Buffer RW	RT	10 ml Add indicated ethanol before first use
RNase-free H2O	RT	6 ml
RNA Bind Columns	RT	50

All reagents, when store in indicated temperature, are stable for 9 months.

❖ Description

The RNApure Total RNA Kit provides a simple, reliable, and rapid method for isolating high-quality total RNA from a variety of samples, including animal and plant cells and tissue. The kit utilizes the strong lysis capability of Buffer RL (TRIzol® Reagent), followed by a convenient and time-saving silica-column purification protocol to purify ultrapure total RNA within an hour.

The sample is first lysed with Buffer RL, according to the lysate preparation protocol provided. The addition of chloroform to your sample, followed by centrifugation separates the solution into an upper aqueous phase containing RNA and a lower phenol-containing organic phase. The upper aqueous phase is transferred to a new tube, followed by ethanol addition and centrifugation. The sample is then transferred to the Spin Column containing a clear silica–based membrane to which the RNA binds during purification. The RNA is washed to remove contaminants and the purified total RNA is then eluted in RNase-Free Water (Tris Buffer, pH 7.5 may also be used) and is suitable for use in a variety of downstream applications including sensitive gene expression studies such as microarray analysis or real time quantitative RT-PCR (qRT-PCR).

❖ Features

1. Combined TRIzol®-based lysis and spin column-based RNA isolation technologies
2. Superior lysis capability and convenient protocol

❖ Note

If your downstream applications require DNA-free total RNA, protocols for

on-column DNase treatment during RNA purification are provided in DNase Digestion Kit (On Spin Columns), Cat# CYRN3401.

❖ **Procedure**

Note：

⇨ **Before the first use, add the indicated amount of ethanol into Wash Buffer RW bottles, mix well, and mark the bottle with a check.**

1. **For Tissues, follow step 1a; for Adherent Cells, follow step 1b; for Suspension Cells, follow step 1c.**

1a. Homogenize tissue samples in 1 mL Buffer RL per 50–100 mg tissue using a tissue homogenizer or rotor-stator. The sample volume should not exceed 10% of the volume of Buffer RL used for homogenization.

1b. Lyse cells directly in a culture dish by adding 1 mL Buffer RL to the dish and passing the cell lysate several times through a pipette tip. The amount of Buffer RL required is based on the culture dish area (1 mL per 10 cm^2) and not on the number of cells present.

1c. Harvest cells and pellet cells by centrifugation. Use 1 mL Carefully remove all supernatant by aspiration. Buffer RL per 5–10 × 10^6 animal, plant, or yeast cells, or per 1 × 10^7 bacterial cells. Lyse cells by repetitive pipetting up and down. Do not wash cells before addition of Buffer RL to avoid any mRNA degradation. Disruption of some yeast and bacterial cells may require a homogenizer.

2. Incubate the lysate with Buffer RL at room temperature for 5 min to allow complete dissociation of nucleoprotein complexes.

3. Add 0.2 mL chloroform or 50 µl 4-Bromoanisole per 1 mL Buffer RL used. Shake the tube vigorously by hand for 15 s.

   Note: Vortexing may increase DNA contamination of your RNA sample. Avoid vortexing if your downstream application is sensitive to the presence of DNA or perform a DNase-digestion step during RNA purification or after purification.

4. Incubate at room temperature for 2–3 min.

5. Centrifuge the sample at 13,000 rpm for 15 min at 4°C.

   Note: After centrifugation, the mixture separates into a lower, red phenol–chloroform phase, an interphase, and a colorless upper aqueous phase which contains the RNA. The volume of the aqueous upper phase is ~500–600 µl.

6. Transfer ~480 µl of the colorless, upper phase containing the RNA to a fresh RNase-free tube. Add an 0.5 volume (240 µl) of ethanol to obtain a final ethanol

concentration of 33%. Pipet to mix immediately. Precipitation may be formed after the addition of ethanol, but this does not affect the procedure. Do not centrifuge.

Note: You can also transfer all upper phase to obtain higher yield.

7. Transfer up to 720 µl mixture into a RNA binding column placed in a 2 ml collection tube (provided). Centrifuge at 13,000 rpm for 30 s, and discard the flow-through. Repeat this step if the sample volume exceeds 720 µl.

   Optional: If your downstream application requires DNA-free total RNA, proceed to On-Column DNase Treatment during RNA Purification at this time (see DNase Digestion Kit manual, Cat# CYRN3401).

8. Add 500 µl Wash Buffer RE, and centrifuge at 12,000 rpm for 30 s. Discard the flow-through.
9. Add 500 µl Wash Buffer RW (with ethanol added), and centrifuge at 12,000 rpm for 30 s. Discard the flow-through. Repeat Step 9 with another 500 µl Wash Buffer RW.
10. Place the RNA binding column back into the same collection tube. Centrifuge the empty column at 13,000 rpm for 2 min to completely remove ethanol from the column.
11. Place the column in a RNase free microcentrifuge tube. Add 30-50 µl of RNase free water (Optional: pre-warm the water to 70–90°C will increase the RNA yield) to the center of the column membrane. Incubate at room temperature for 1 min, and centrifuge at 12,000 rpm for 1 min to elute the RNA.

    Note: If the expected RNA yield is >30 µg and want higher yield, repeat step 11 with another 30–50 µl of RNase-free water; if want higher concentration, repeat step 11 using the eluate from step 11. Reuse the centrifuge tube from step 11.

12. Store the purified RNA on ice if used within a few hours. For long-term storage, store the purified RNA at –80°C.

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