CHANGYU Plus RNA Mini Kit (Cells or Tissues)

Cat#: CYRN2802 (50 T)

Kit Contents	Storage	50 Preps (CYRN2802)
Buffer RLT Plus	RT	50 ml
Buffer RW1	RT	40 ml
Wash Buffer RW	RT	10 ml *Add indicated ethanol before first use*
70% Ethanol	RT	9 ml RNase free H_2O *Add indicated ethanol before first use*
RNase-free H_2O	RT	10 ml
Genomic DNA Elimination Columns	RT	50
RNA Binding Columns	RT	50

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

Description

The innovative CHANGYU plus system is ideal or rapid purification of genomic DNA-free total RNA from animal cells or tissues. The unique genomic DNA elimination columns effectively remove genomic DNA from RNA samples. No DNase digestion step is required. The purified total RNA can be directly used for reverse transcription and RT-PCR. The whole process is phenol/chloroform-free. The unique lysis buffer immediately lyses biological samples and inactivates RNase and DNase. The lysate containing RNA is then passed through a genomic DNA elimination column, where genomic DNA binds to the column membrane, and RNA remains in the flow-through. Ethanol is added to the flow-through to provide appropriate binding conditions for RNA, and RNA selectively binds to the silica-membrane of the RNA column in the high-salt buffer. RNA is purified through a series of wash-spin steps to remove protein followed by elution of RNA from silica-membrane with RNase-free H_2O.

❖ Features

1. Unique gDNA Elimination columns avoid the need for DNase
2. Efficient removal of genomic DNA
3. Highly reproducible yields of RNA in minutes

4. High-performance RNA for sensitive applications

Procedure

Note：

⇨ **Before the first use, add the indicated amount of ethanol into Wash Buffer RW and 70% ethanol bottles, mix well, and mark the bottle with a check.**

1. **Culture cells:**

a. Harvest <10^7 cells grown in suspension into a centrifuge tube. Adherent cells can be lysed directly in cell-culture vessels or trypsinized from culture flasks and collected into a centrifuge tube.

b. Centrifuge at 13,000 rpm for 10 sec (or 800 rpm for 5min) to pellet cells. Completely aspirate the supernatant.

Note: Incomplete removal of the supernatant will decrease the yield and purity.

c. Loosen the cell pellet thoroughly by flicking the tube. Add 350 µl (<$5x10^6$ cells) or 600 µl ($5x10^6$-$1x10^7$ cells) of Buffer RLT Plus, pipet or vortex to mix.

d. Homogenization: (cells <$1x10^5$ can be homogenized by vortexing for 1 min.) Pass the lysate at least 5 times through a 20-gauge needle (0.9 mm diameter) fitted to an RNase-free syringe. Homogenization shears genomic DNA, reduces the viscosity of the lysates, and increases the yields.

e. Apply the homogenized lysate to a DNA elimination column placed in a 2 ml collection tube (supplied).

f. Immediately proceed to Step 3.

2. **Animal tissues (for example, mouse liver and brain):**

a. Mince fresh tissues into small pieces, add 350 µl (<20 mg tissue) or 600 µl (20-30mg tissue) of Buffer RLT Plus. Homogenize with electronic tissue homogenizer for 20-40 s. Or

b. Immediately place the tissue in liquid nitrogen, and grind thoroughly with a mortar and pestle. Transfer adequate amount (20mg/30mg) of tissue powder in to a 1.5 ml microcentrifuge tube containing 350 µl/600 µl of Buffer RLT Plus, vortex for 20 s. Pass the lysate at least 5 times through a 20-gauge needle (0.9 mm diameter) fitted to a syringe or homogenize with an electronic tissue homogenizer. This step shears genomic DNA, reduces the viscosity of the lysates, and increases the yields.

c. Centrifuge the homogenized lysate at 13,000 rpm for 3 min. Transfer the supernatant into a DNA elimination column carefully in a provided 2 ml collection tube.

d. Immediately proceed to Step 3.

3. Centrifuge at 13,000 rpm for 60 s, and save the flow-through (RNA is in the flow-through). Ensure all the samples flow through the column. Increase centrifuge speed and time, if necessary.
4. Add equal volume of 70% ethanol (usually 350 µl to 600 µl, adjust the ethanol volume accordingly if some lysate is lost during the above procedure) and pipet to mix immediately. Precipitation may be formed after the addition of ethanol, but this does not affect the procedure. Do not centrifuge.
5. Transfer up to 700 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 700 µl.
6. Add 700 µl Buffer RW1, and incubate at room temperature for 1 min. Centrifuge at 12,000 rpm for 30 s. Discard the flow-through.
7. Add 500 µl Wash Buffer RW, and centrifuge at 12,000 rpm for 30 s. Discard the flow-through. Repeat Step 7 with another 500 µl Wash Buffer RW.
8. 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.
9. Place the column in a RNase free centrifuge 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.
10. If the expected RNA yield is >30 µg, repeat step 9 with another 30–50 µl of RNase-free water, or using the eluate from step 9 (if high RNA concentration is required). Reuse the centrifuge tube from step 9.
    If using the eluate from step 9, the RNA yield will be 15–30% less than that obtained using a second volume of RNase-free water, but the final RNA concentration will be higher.

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