

RNAprep Micro Sample Total RNA Kit

(Spin-column)

RP 5100 (20 preps)

RP 5101 (50 preps)

Kit Content, Storage Condition and Stability

Content	Storage	20 preps (RP 5100)	50 preps (RP 5101)
Buffer RL	4°C	25 ml	55 ml
Carrier	-20°C	300 ul	600 ul
Buffer RE	RT	15 ml	30 ml
Buffer RW	4 °C (half 1 year) -20 °C (long term)	6 ml Add ration ethanol before first use	15 ml
RNase-free H ₂ O	RT	5 ml	10 ml
RNase-free Spin-column AC	RT	20	50
Collection Tube (2ml)	RT	20	50
70% ethanol	RT	4 ml RNase-free H ₂ O Add ration ethanol before first use	9 ml RNase-free H ₂ O

All reagents, when stored properly, are stable for 12 months.

Notes:

Please add ration ethanol to Buffer RW and 70% ethanol before first use. Mix well and mark the check box labeled on the bottles to indicate that the ethanol has been added.

All reagents should be clear. In case, some may precipitate due to low temperature, please incubate them at 37°C for a moment **until clear**, and then cool down to RT before use.

Some reagents will precipitate because of been stored at 4°C or -20°C, which will affect the using effect, so incubate before use till no pricipation is visible. All reagents can be transported under room temperature (15°C - 25°C). Buffer RL can be transported under RT and kept at 4°C upon arrival.

Please ensure the bottles tightly capped when not in use, preventing reagents from evaporating, oxidation and pH change.

Principle

This technology combines the selective binding properties of a silica membrane with the speed of microspin technology. Biological samples are first lysed and homogenized in the presence of a highly denaturing guanidine-thiocyanate-containing buffer, which immediately inactivates RNases to ensure intact RNA. Ethanol is added to provide appropriate binding conditions, and the sample is then applied to an RNAPure spin-column, where the total RNA binds to the membrane and contaminants are efficiently washed away. Carrier has been used in this kit to increase micro-scale RNA precipitation. High-quality RNA is then eluted in 20-50µl water.

Features

Stability, comparable RNA yield with high quality absorbing membrane.

High-purity, specifically membrane absorption and washing for removing protein and other debris.

Special Buffer RL removes genomic DNA

Carrier facilitates micro scale total RNA precipitation

Notes

To prevent RNA degradation, all the centrifuge steps should be made under 4°C, except having special notes.

Buffer RL and Buffer RE contain stimulating compound, please wear latex gloves, avoiding skin, eyes and cloth to be contaminated. If that, please use water or physiological saline to wash the exposed body parts.

Due to the prevalence of RNases, wear gloves at all times and change them whenever may have been contacted by reagents, please follow standard laboratory procedures of "Molecular Clone" rules.

** Wear gloves in entire process. Skin often contains bacteria and molds that can contaminate an RNA preparation and be a source of RNases.*

** Use sterile, disposable plasticware and automatic pipettes reserved for RNA work to prevent cross-contamination with RNases from shared equipment. For example, a laboratory that is using RNA probes will likely be using RNase A or T1 to reduce background on filters, and any nondisposable items (such as automatic pipettes) can be rich sources of RNases.*

** Treat non-disposable glassware and plastic ware before use to ensure that it is RNase-free. Bake glassware at 200°C overnight, and thoroughly rinse plastic ware with 0.1N NaOH, 1mM EDTA followed by RNase-free water.*

The integrity of purified RNA may be determined by denaturing agarose gel electrophoresis (or agarose gel electrophoresis). The ratio of 28S to 18S ribosomal RNA should be approximately 2:1 by ethidium bromide staining. Sometimes there may be the third band about 0.1-0.3kb (5S RNA and tRNA), even 4 or 5 bands will appear in some plant tissues. Once the preRNA, hnRNA, small RNA are extracted from the sample, you will see some discontinuous bands of 7kb-15kb. All of them are normal.

The most common method to determine the yield and purity of RNA is spectrophotometry (OD260/OD280). Please dissolve RNA by TE, water will make OD280 higher because of lower ion intensity and pH.

Prepare chloroform before use.

Sample lysate in Buffer RL is stable up to one month at -70°C.

Procedure

Note: Add absolute ethanol to Buffer WB and 70% ethanol. Prepare chloroform before use.

1. Homogenization:

a. Tissues

Please homogenize tissue in an appropriate volume of Buffer RL (50-100mg/mL) until no visible tissue. Pay attention to the volume of sample should not be beyond 1/10 total volume of Buffer RL.

b. Cells Grown in Monolayer

You can directly append an appropriate volume Buffer RL to the culture plate for dissolving cells and transfer dissolution by pipetting. The volume of Buffer RL is decided by the area of culture plate, about 10 cm² per 1 ml. Once appending not enough Buffer RL, it's possible to contaminated genomic DNA.

c. Cells Grown in Suspension

Pellet cells by centrifugation. Lyse cells in RL by repetitive pipetting. Use 0.75 ml of the reagent per 5-10 × 10⁶ of animal, plant or yeast cells, or per 1 × 10⁷ bacterial cells. Washing cells before addition of RL should be avoided as this increases the possibility of mRNA degradation. Disruption of some yeast and bacterial cells may require use of a homogenizer.

2. Incubate the homogenized samples for 5 minutes at 15 - 30°C to permit the complete dissociation of nucleoprotein complexes.

3. Optional point: Centrifuge at 12,000 rpm for 10 min at 4°C. Pipette the supernatant to a RNase-free centrifuge tube. *An additional isolation step may be required for samples with high content of proteins, fat, polysaccharides or extracellular material such as muscles, fat tissue and tuberous parts of plants.*

4. Add 0.2 ml of chloroform per 1 ml of Buffer RL. Cap sample tubes securely. Shake tubes vigorously by hand for 15 sec and incubate them at 30°C for 2 - 3 minutes.
5. Centrifuge the samples at 12,000 rpm for 10 minutes at 4°C.
6. Transfer the aqueous phase to a fresh tube. Add 1 volume 70% ethanol and 6 ul of Carrier. Mix well (precipitate may form). Transfer the mixture and precipitate to a Spin-column AC (placed in collection tube). If the mixture is too much, apply the mixture in successive application to the same Spin-column AC.
7. Centrifuge at 10,000 rpm for 45 sec at 4°C. Discard the flow through. Reuse the Spin-column and the collection tube.
8. Add 500µl Buffer RE to the center of Spin-column AC to remove proteins. Centrifuge at 12,000 rpm for 45 sec. Discard the flow through.
9. Add 700µl Buffer RW. Centrifuge at 12,000 rpm for 45 sec. Discard the flow through.
10. Add 500µl Buffer RW. Centrifuge at 12,000 rpm for 45 sec. Discard the flow through.
11. Place Spin-column AC to the collection tube and spin for 2 min to remove the residual fluid.
12. Place Spin-column AC to a 1.5 ml RNase-free centrifuge tube. Apply 20-50µl RNase-free H₂O (pre-heated to 65°C - 75°C is better) to the center of the column. Place it at room temperature for 2 min. Centrifuge at 12,000rpm for 1 min. If desired, wash the Spin-column AC with 30 µl RNase-free water, combining the second eluate with the first in the same Collection Tube; approximately 90% of the RNA is recovered during the first elution step.

Problem	Possible Reason	Advices
Low RNA yield	Incomplete lysis and homogenization	Decrease the amount of starting material used, or increase volume of Lysis Buffer. Use the proper homogenization methods according to recommendations in the sample-specific protocols. Cut tissue samples into smaller pieces and ensure the tissue is completely immersed in the Lysis Buffer to achieve optimal lysis.
	Poor quality of starting material	The yield and quality of RNA isolated depends on the type and age of the starting material. Be sure to use fresh sample and process immediately after collection or freeze the sample at -80°C or in liquid nitrogen immediately after harvesting.
	Beyond the binding maximum of silica membrane RNA	Use multiple Spin-columns AC for the same sample.
	Ethanol not added to Wash Buffer RW	Be sure that ethanol was added to Wash Buffer RW
	Carrier not added	Add Carrier in the step 6
Inhibition of downstream enzymatic reactions	Presence of ethanol in purified RNA	Place the Spin column into the Wash tube and centrifuge the spin column at maximum speed for 2-3 min to completely dry the cartridge
Low A260/280 ratio	Sample was diluted in water. Non-buffered water has variable pH	Use 10mM Tris-HCl (pH 7.5) to dilute sample for OD measurements.
RNA degraded	RNA contaminated with RNase	Use RNase-free pipet tips with aerosol barriers. Change gloves frequently.
	Improper handling of sample from harvest until lysis	If not processed immediately, quick-freeze tissue immediately after harvesting and store at -80°C or in liquid nitrogen. Frozen samples must remain frozen until Lysis Buffer was added. Perform the lysis quickly after adding Lysis Buffer.