Note: for laboratory research use only

Genomic DNA and Total RNA Separate Extraction Kit (Spin-column）

Cat. #: RP5501 (50 preps)

Signalway Biotechnology

Ⅰ.Kit Content, Storage, and Stability

Content	Storage	50 preps (RP5501)
Buffer DLS	4℃	55 ml
Buffer RE	RT	60 ml
Buffer RW	4℃ (half year) -20℃ (long term)	25 ml
		Add 100 ml ethanol before use
RNase-free H_2O	RT	20 ml
Protein Precipitation Buffer	RT	30 ml
RNase-free Spin-column RA	RT	50
RNase-free Spin-column RB	RT	50
Collection Tube (2ml)	RT	100 pcs
Buffer RH	RT	40 ml RNase-free H_2O
		Add 95 ml ethanol before use

All reagents are stable for 18 months at RT, when stored properly.

Reminder:

1 · ***Add ethanol to Buffer WB and Buffer RH before use; mix adequately, and then check the box on the label showing ethanol has been added!***

2 · All reagents should be clear. Some precipitation may occur under low temperature. Incubate the buffer at 37°C for a few minutes **until buffer is clear**, and then let the buffer cool to RT before use.

3 · Keep the reagent lids tightly caped when not in use to prevent evaporation, oxidation, and changes in pH.

Ⅱ.Principle:

Biological samples are lysed and homogenized in the presence of a highly denaturing guanidine-thiocyanate–containing buffer, which immediately inactivates RNases to ensure intact RNA. Buffer RH with ethanol is used to provide appropriate binding conditions, and mixture is then applied to the first spin-column, where genomic DNA binds to the membrane. Then add filtrate to the second spin-column, and total RNA binds to this membrane. Finally, contaminants are efficiently removed separately. High-quality genomic DNA and total RNA are then eluted in 30-100 μl water from separate spin-column.

Ⅲ.Features:

1・Excellent yield.

2・High-purity; unique membrane absorption and specialized washing for removing protein and other debris.

Ⅳ.Notes

Read this section before your experiment.

1・ **To prevent RNA degradation, all the centrifugation steps should be performed under 4℃ unless being specially noted**. Recommend using traditional centrifuge (with up to 13,000 rpm), such as Eppendorf 5415C.

2・ Buffer DLS and Buffer RE contains a corrosive compound; please wear latex gloves to avoid direct contact with skin, eyes, and clothes**. If contact occurs, wash with water or physiological saline.**

3・ Due to the prevalence of RNases, wear gloves during the procedure and change them whenever contact by reagents occurs; please follow standard laboratory procedures of “Molecular Clone” rules.

* *Wear gloves during entire process. Skin often contains bacteria and molds*

that can contaminate an RNA preparation and be a source of RNases.

** Use sterile and disposable plastic ware and automatic pipettes reserved for RNA work to prevent cross-contamination with RNases from shared equipment. For example, a laboratory using RNA probes likely has been 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 non-disposable plastic-ware before use to ensure that they are RNase-free. Bake glassware at 200°C overnight, and thoroughly rinse plastic-ware with 0.1N NaOH and 1mM EDTA followed by RNase-free water.*

4 · 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. There may be the third band about 0.1-0.3 kb (5S RNA and tRNA), or even 4 or 5 bands appear from purified RNA from some plant tissues. The preRNA, hnRNA, and small RNA appear as some bands at sizes between 7-15 kb. All of them are normal.

5 · The most common method to determine the yield and purity of DNA or RNA is spectrophotometry (OD_{260}/OD_{280}). Dissolve DNA and RNA by TE (water will make OD_{280} higher because of lower ion intensity and pH).

Ⅴ.Procedure

Before Starting

- ***Add absolute ethanol to Buffer WB and Buffer RH!***

1 Homogenization:

a. **Tissues**

Please homogenize tissue in an appropriate volume of Buffer DLS (50-100 mg/ml) until no visible tissue. The volume of sample should be no more than 1/10 of total volume of Buffer DLS.

b. **Cells Grown in Monolayer**

Use an appropriate volume Buffer DLS to the culture plate for suspending cell and transfer by pipetting. The volume of Buffer DLS (about 10 cm^2 per 1 ml) is determined by the area of culture plate. Use enough Buffer DLS to avoid low-yield or contaminated genomic DNA.

c. **Cells Grown in Suspension**

Pellet cells by centrifugation. Lyse cells in Buffer DLS by repeating pipetting. Use 0.75 ml of the buffer per 5-10 × 10^6 animal, plant or yeast cells, or per 1 × 10^7 bacterial cells. Washing cells before adding Buffer DLS should be avoided because this increases the possibility of mRNA degradation. Disruption of some yeast and bacterial cells may require the use of a homogenizer.

2 Incubate the homogenized samples for 10 minutes at 37°C to permit the complete dissociation of nucleoprotein complexes.

3 Add 1/3 volume Protein Precipitation Buffer and incubate on ice-water for 8 min.

4 Centrifuge at 12,000 rpm for 10 minutes at 4℃. Pipette the supernatant to an RNase-free Spin-column RA.

5 Centrifuge at 12,000 rpm for 1 minute. Transfer Spin-column RA (contains genomic DNA) to another Collection Tube. **Please follow steps 8-13 to purify genomic DNA.**

6 Add 1 volume Buffer RH to the filtrate from step 5 and mix gently. Transfer the mixture to a Spin-column RB.

7 Centrifuge at 10,000 rpm for 45 seconds, and discard filtrate.

Please centrifuge twice if the volume of mixture is more than 700 µl.

8 Add 500 µl Buffer RE to the center of Spin-column RB (or RA), centrifuge at 12,000 rpm for 45 seconds. Discard the filtrate.

9 Add 700 µl Buffer RW (**check whether ethanol is added!**). Centrifuge at 12,000 rpm for 45 seconds. Discard the filtrate.

10 Add 500 µl Buffer RW. Centrifuge at 12,000 rpm for 45 seconds. Discard the filtrate.

11 Place the Spin-column RB (or RA) back to the Collection Tube and spin at 12,000 rpm for 2 minutes to remove the residual fluid.

12 Place Spin-column RB (or RA) to a 1.5 ml RNase-free centrifuge tube. Apply 50-80 µl RNase-free H_2O (Pre-heated to 65-75 ℃ for a higher yield) to the center of the Spin-column RB (or RA). Place it at room temperature for 2 minutes. Centrifuge at 12,000 rpm for 1 min. If desired, reapply the Spin-column RB (or RA) with 30 µl RNase-free water and combine the second elute with the first flow-through in the same tube; approximately 90% of genomic DNA and total RNA are recovered during the first elution step.

13 Store genomic DNA and total RNA at -20℃ or apply for down-stream reactions.

Ⅶ.Troubleshooting

Problem	Possible Reason	Advices
Low DNA/RNA yield	Incomplete lysis and homogenization	Decrease the amount of starting material, 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 that the tissue is completely immersed in Lysis Buffer to achieve optimal lysis.
	Poor quality of starting material	The yield and quality of isolated RNA depend on the type and qulity of the starting material. Be sure to use fresh sample and process immediately after collection. Samples should be stored at –80 °C or in liquid nitrogen immediately after harvesting.
	Beyond the binding capacity of silica membrane	Use multiple spin-columns (RA or RB) for the same sample.
	Ethanol was not added to Wash Buffer RW	Be sure that ethanol was added to Wash Buffer RW
Low A260/280 ratio	Sample was diluted in water. Non-buffered water has variable pH	Use 10 mM 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 processing immediately, quickly freeze tissue immediately after harvesting and store them at –80°C or in liquid nitrogen. Tissues must remain frozen until Lysis Buffer is added. Perform the lysis quickly after adding Lysis Buffer.
	Tissue very rich in RNases	RNA isolated from tissue rich in RNases may require the addition of RNase inhibitors/inactivators to protect the RNA from degradation or using a larger volume of Lysis Buffer. Elute samples in 100% formamide. If RNA is used for mRNA isolation of Northern blots, elute in 0.1% SDS.

Inhibition of downstream enzymatic reactions	Presence of ethanol in purified RNA	Place Spin column into the Wash Tube and centrifuge the spin column at maximum speed for 2-3 minutes to completely dry the cartridge.