Genomic DNA and Total RNA Separate Extraction Kit

(Spin-Column)

DP 5501 (50 preps)

Kit Content, Storage Condition and Stability

Content	Storage	50 preps
Buffer DLS	4°C	55 ml
Buffer RE	RT	60 ml
Buffer RW	4°C (half year) -20°C (long term)	25 ml Add the ration ethanol before use
RNase-free H_2O	RT	20 ml
RNase-free Spin Column RA	RT	50
RNase-free Spin Column RB	RT	50
Collection Tubes 2 ml	RT	100
70% ethanol (Buffer RH)	RT	45 ml RNase-free H_2O Add the ration ethanol before use

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

Note:

Please add ration ethanol to Buffer RW and 70% ethanol before 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 in 4°C or - 20°C, which will affect the using effect, so incubate till no pricipitation before use. All reagents can be transported under room temperature (15°C-25°C).

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

Principle

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 mixture is then applied to the first spin-column, where genomic DNA binds to the membrane. Then, added filtrate to the second spin-column binds total RNA to the membrane. Finally, contaminants are efficiently washed away separately. High-quality gDNA and total RNA are then eluted in 30-100 µl water from separate spin-column.

Features

- ✓ Stability, comparable RNA yield with high quality absorbing membrane.
- ✓ High-purity, specifically membrane absorption and washing for removing protein and other debris.
- ✓ No protein contamination.

Notes

1. **To prevent RNA degradation, all the centrifugation steps should be performed under 4°C, except having special notes**, using up to 13,000 rpm traditional centrifuge.

2. Buffer DSL 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.**

3. 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 Cloning" rules.
* *Wear gloves in entire process. Skin often contains bacteria and molds that can contaminate RNA preparation and are 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.*

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. Sometimes there may be the third band about 0.1-0.3 kb (5S RNA and tRNA), even 4 or 5 bands will appear in some plant tissues. Once the preRNA, hnRNA, small RNA is extracted from the sample; you will see some discontiguous bands of 7 kb-15 kb. All of them are normal.

5. The most common method to determine the yield and purity of DNA and RNA is spectrophotometry (OD_{260}/OD_{280}). Please dissolute DNA and RNA by TE, water will make OD280 higher because of lower ion intensity and pH.

Procedure

Note: Add absolute ethanol to Buffer WB and 70% ethanol.

1. Homogenization:

a. Tissues

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

b. Cells Grown in Monolayer

You can directly append an appropriate volume Buffer DLS to the culture plate for dissolve cell and transfer dissolution by pipetting. The volume of Buffer DLS is decided by the area of culture plate, about $10cm^2$ per 1 ml. Once appending not enough Buffer DLS, it's possible to contaminated genomic DNA.

c. Cells Grown in Suspension

Pellet cells by centrifugation. Lyse cells in DLS by repetitive pipetting. Use 0.75 ml of the reagent per 5-10 × 10^6 of animal, plant or yeast cells, or per 1 × 10^7 bacterial cells. Washing cells before addition of DLS should be avoided as 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. Centrifuge at 12,000 rpm for 10 min at 4°C. Pipette the supernatant to an RNase-free Spin-column RB, centrifuge at 12,000 rpm for 1 min at 4°C.

Choose: Place Place spin-column RB (contains genomic DNA) to a new collection tube and follow steps 6 - 11 to purify genomic DNA.

Steps 4 - 11 are for purifying of total RNA

4. Collect the filtrate from steps 3 to a new collection tube and add 1 volume 70% (check ethanol added!), mix gently. Transfer the mixture to a Spin-column RA.

5. Centrifuge at 10,000 rpm for 45 sec, discard filtrate.

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

7. Add 700µl Buffer RW (check ethanol added!). Centrifuge at 12,000 rpm for 60 sec. Discard the filtrate.

8. Add 500 µl Buffer RW. Centrifuge at 12,000 rpm for 60 sec. Discard the filtrate.

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

10. 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°C-75°C is better) to the center of the Spin-column RB (or RA). Place it at room temperature for 2 min and centrifuge at 12,000 rpm for 1 min. If desired, wash the Spin-column RB (or RA) with 30 µl RNase-free water, combining the second eluate with the first in the same tube; approximately 90% of genomic DNA and total RNA are recovered during the first elution step.

11. Please store genomic DNA and total RNA at -20°C and -80°C for long-term storage.

Troubleshooting

Problem	Possible Reason	Advices
Low DNA/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 maxium of silica membrane RNA	Use multiple spin-columns RA for the same sample
	Ethanol 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 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.
	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 use a larger volume of Lysis Buffer. Elute samples in 100% formamide. If the 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 the Spin column into the Wash Tube and centrifuge the spin column at maximum speed for 2-3 minutes to completely dry the cartridge.

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