RNAprep Pure Tissue Kit

For purification of total RNA from animal tissue

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RNAprep Pure Tissue Kit

(Spin Column)

Cat. no. GDP431

Kit Contents

	Contents	GDP431 (50 preps)
GDP431H	Buffer RL	30 ml
	Buffer RW1	40 ml
	Buffer RW	12 ml
	Proteinase K	500 μl
	Grinding Pestles	10
	RNase-Free ddH_2O	40 ml
	RNase-Free Columns CR3 set	50
	RNase-Free Centrifuge Tubes (1.5 ml)	50
	Handbook	1
GRT411	RNase-Free DNase I (1500 U)	1
	Buffer RDD (DNA Digest Buffer)	4 ml
	RNase-Free ddH_2O	1 ml

Note: GDP431H, GRT411 are shipped and packaged separatly.

Storage

RNase-free DNase I, Buffer RDD and RNase-free ddH_2O (Tubular) should be stored at 2-8°C for 15 months; Buffer RL/β-mercaptoethanol mix can be stored at 2-8°C for 1 month; others stored at room temperature (15-30°C) for 15 months.

Introduction

RNAprep Pure Tissue Kit provides a fast, simple, and cost-effective method for purification of total RNA from animal tissues. The purified RNA is ready for use in downstream applications such as RT-PCR, real-time RT-PCR, gene-chips assay, northern blot, dot blot, polyA screening, in vitro transcript, molecular cloning and other downstream applications.

Notes of preventing RNase contamination

1. Change gloves regularly. Bacteria on the skin can result in RNase contamination.
2. Use RNase-free plastic and tips to avoid cross-contamination.
3. RNA can be protected in Buffer RL. But RNA must be stored or applied in RNase-free plastic or glassware. To wipe off RNase, the glassware can be roasted at 150°C for 4 hours, while plastic can be dipped in 0.5 M NaOH for 10 min, washed by RNA-free ddH_2O thoroughly and sterilized.
4. Use RNase-free ddH_2O to confect solution.

Important notes before starting

1. β-Mercaptoethanol (β-ME) must be added to Buffer RL before use. The final concentration of β-ME is 1%. For example, add 10 μl β-ME to 1 ml Buffer RL. Buffer RL containing β-ME can be stored at 2-8°C for 1 month. Buffer RL may form a precipitate upon storage. If necessary, redissolve by warming, and then place at room temperature (15–30°C).
2. Buffer RW is supplied as a concentrate. Before using for the first time, add ethanol (96–100%) as indicated on the bottle to obtain a working solution.
3. Perform all steps in RT if not showed.

Preparation of DNase I stock solution

Dissolve the lyophilized DNase I (1500 units) in 550 μl of the RNase-free ddH_2O. Mix gently by inverting. Do not vortex. Divide it into single-use aliquots, and store at -30~-15°C for up to 9 months. Thawed aliquots can be stored at 2–8°C for up to 6 weeks. Do not refreeze the aliquots after thawing.

Protocol

1. Disrupt the tissue and homogenize the lysate.

   Place the 10-20 mg tissue in liquid nitrogen immediately, and add 300 µl Buffer RL **(Ensure that β-ME has been added to Buffer RL before use).** Grind tissue thoroughly with a pestle. Add 590 µl RNase-free ddH_2O to the lysate. Then add 10 µl proteinase K solutions, and mix thoroughly by pipetting. Incubate at 56°C for 10-20 min.

   Note: Determine the amount of tissue. Do not use more than 20 mg.

2. Centrifuge for 2-5 min at 12,000 rpm (~13,400 × g). Carefully transfer the supernatant to a new microcentrifuge tube (not supplied).
3. Add 0.5 volume of ethanol to the cleared lysate, and mix immediately by pipetting. Transfer the sample, including any precipitate that may have formed, to RNase-free Spin Column CR3 placed in a 2 ml RNase-free Collection Tube. Close the lid gently, and centrifuge for 30-60 s at 12,000 rpm (~13,400 × g). Discard the flow-through.
4. Add 350 µl Buffer RW1 to the RNase-free Spin Column CR3 Close the lid gently, and centrifuge for 30-60 s at 12,000 rpm (~13,400 × g). Discard the flow-through.
5. Preparation of DNase I working solution: Add 10 µl DNase I stock solution (see Preparation of DNase I stock solution) to 70 µl Buffer RDD. Mix by gently inverting the tube.
6. Add the DNase I working solution (80 µl) directly to the RNase-free Spin Column CR3, and place on the bench top for 15 min.
7. Add 350 µl Buffer RW1 to the RNase-free Spin Column CR3. Close the lid gently, and centrifuge for 30-60 s at 12,000 rpm (~13,400 × g). Discard the flow-through.
8. Add 500 µl Buffer RW to the RNase-free Spin Column CR3. **(Ensure that ethanol has been to added Buffer RW before use)** Close the lid gently, incubate for 2 min at room temperature and centrifuge for 30-60 s at 12,000 rpm (~13,400 × g). Discard the flow-through.
9. Repeat step 8.
10. Centrifuge for 2 min at 12,000 rpm (~13,400 × g) to dry the spin column membrane.

    Note: The long centrifugation dries the spin column membrane, ensuring that no ethanol is carried over during RNA elution. Residual ethanol may interfere with downstream reactions.

11. Place the RNase-free Spin Column CR3 in a new 1.5 ml RNase-free Collection Tube (supplied). Add 30-100 µl RNase-free ddH_2O directly to the centre of spin column membrane. Place at RT for 2 min, close the lid gently, and centrifuge for 2 min at 12,000 rpm (~13,400 × g) to elute the RNA.

    Note: Volume of elution buffer should no less than 30 µl, or else it will reduce elution efficiency. Purified RNA may be stored at –70°C.