RNAprep Pure Tissue Kit

For purification of total RNA from animal tissue, cultured cell and bacteria

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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.

Compatible Reagents

Lysozyme (self provide, TIANGEN, Cat. no GRT401-11, for bacteria RNA purification)

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 tissue, cultured cell and bacteria. 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 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.
4. Do not recommend storage sample in buffer with lysing capacity.

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

Purification of total RNA from tissue

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.

Purification of total RNA from cultured animal cells

1. Harvest cells:

   a. Cells grown in suspension (do not use more than 1 x 10^7 cells):

   Determine the number of cells. Pellet the appropriate number of cells by centrifuging for 5 min at 300 x g in a centrifuge tube (not supplied). Carefully remove all supernatant by aspiration.

   b. Cells grown in a monolayer (do not use more than 1 x 10^7 cells):

   Cells can be either lysed directly in the cell-culture vessel (up to 10 cm diameter) or trypsinized and collected as a cell pellet prior to lyses. Cells grown in cell-culture flasks should always be trypsinized.

   (1) To lyse cells directly

   Determine the number of cells. Completely aspirate the cell-culture medium, and proceed immediately to step 2.

   (2) To trypsinize and collect cells

   Determine the number of cells. Aspirate the medium, and wash the cells with PBS. Aspirate the PBS, and add 0.10-0.25% trypsin in PBS. After the cells detach from the dish or flask, add medium (containing serum to inactivate the trypsin), transfer the cells to an RNase-Free glass or polypropylene centrifuge tube (not supplied), and centrifuge at 300 × g for 5 min. Aspirate the supernatant completely.

   Note: Incomplete removal of cell-culture medium will inhibit lysis and dilute the lysate, affecting the conditions for binding of RNA to the spin column membrane. Both effects may result in lower RNA yield.

2. Disrupt the cells by adding Buffer RL

   For pelleted cells, loosen the cell pellet thoroughly by flicking the tube. Add the appropriate volume of Buffer RL **(see the table as below; ensure that β-Mercaptoethanol has been added to Buffer RL before use).**

Number of pelleted cells	Volume of Buffer RL (μl)
$< 5 \times 10^6$	350
5×10^6 - 1×10^7	600

For direct lysis of cells grown in a monolayer, add the appropriate volume of Buffer RL **(see the table as below, ensure that β-Mercaptoethanol has been added to Buffer RL before use)**. Pipet the lysate into a centrifuge tube (not supplied). Vortex or pipet to mix, and ensure that no cell clumps are visible before proceeding to step 3.

Volumes of Buffer RL for Direct Cell Lysis

Dish diameter (cm)	Volume of Buffer RL (μl)
< 6	350
6-10	600

3. Add 10 μl proteinase K solutions, and mix thoroughly by pipetting.
4. Add 1 volume of 70% ethanol (usually 350 μl or 600 μl) 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.

   Note: please confect 70% ethanol with RNase-Free ddH_2O. If sample is lost partly, reduce accordingly the amount of 70% ethanol; if lysate volume exceeds 700 μl, centrifuge successive aliquots in the same spin column.
5. 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.
6. 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. Inverting the tube gently to mix.
7. Add the DNase I working solution (80 μl) directly to the center of RNase-Free Spin Column CR3, and place on the bench top for 15 min.

8. 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.
9. Add 500 µl Buffer RW to the RNase-Free Spin Column CR3 (**Ensure that ethanol has been added to Buffer RW before use**). Close the lid gently, place in room temperature for 2 min and centrifuge for 30-60 s at 12,000 rpm (~13,400 × g). Discard the flow-through.
10. Repeat step 9.
11. Centrifuge at 12,000 rpm (~ 13,400 × g) for 2 min, and then discard the waste liquid. Place the RNase-Free Spin Column CR3 at room temperature for 2-5 mins to completely dry the residual washing buffer in the column.
    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.
12. 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 spin column membrane. Close the lid gently, place at room temperature (15-30°C) for 2 min and centrifuge for 2 min at 12,000 rpm (~13,400 × g) to elute the RNA.
    Note: If the volume of elution buffer is less than 30 µl, it may affect recovery efficiency. Purified RNA is recommended be stored at -70°C.

Purification of total RNA from bacteria

1. Collect bacteria by centrifuging for 12,000 rpm (~13,400 × g) at 4°C in a centrifuge tube (do not use more than 1 x 10^9 cells). Carefully remove all supernatant by aspiration, and proceed to step 2. Other steps should be completed at RT (15-30°C).
   Note: non-complete removal of culture medium will inhibit lysis and dilute the lysate.
2. Re-suspend bacteria thoroughly with 100 µl TE buffer containing lysozyme (recipe and incubation time have been showed as follow).

	Concentration of lysozyme in TE buffer	Incubation time (RT)
G- bacterium	400 µg /ml	3-5 min
G+ bacterium	3 mg/ml	5-10 min

3. Add 350 µl Buffer RL **(Ensure that β-Mercaptoethanol has been added to Buffer RL before use)**. Vortex or pipet to mix, and ensure that no cell clumps are visible before proceeding to step 4. If insoluble precipitate has been formed, centrifuge for 2 min at 12,000 rpm (~13,400 × g). Transfer supernatant to another centrifuge tubes.

4. Add 250 µl ethanol to the cleared lysate, 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, and put the spin column back to the Collection Tube.
5. 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.
6. 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. Inverting the tube gently to mix.
7. Add the DNase I working solution (80 µl) directly to the center of RNase-Free Spin Column CR3, and place on the bench top for 15 min.
8. 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.
9. Add 500 µl Buffer RW to RNase-Free Spin Column CR3 **(Ensure that ethanol has been added to Buffer RW before use)**. Close the lid gently, place in room temperature for 2 min and centrifuge for 30-60 s at 12,000 rpm (~13,400 × g). Discard the flow-through.
10. Repeat step 9.
11. Centrifuge at 12,000 rpm (~13,400 × g) for 2 min, and then discard the waste liquid. Place the RNase-Free Spin Column CR3 at room temperature for 2-5 mins to completely dry the residual washing buffer in the column.
    Note: long centrifugation could dry the spin column membrane, ensuring that no ethanol is carried over during RNA elution. Residual ethanol may interfere with downstream reactions.
12. 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 spin column membrane. Close the lid gently, place at room temperature (15-30°C) for 2 min and then centrifuge for 2 min at 12,000 rpm (~13,400 × g) to elute the RNA.
    Note: If the volume of elution buffer is less than 30 µl, it may affect recovery efficiency. Purified RNA is recommended be stored at -70°C.