

Ver. 24111

ODP419

Total RNA Kit

For extraction of total RNA from blood, cells, fungus, tissues, serum and plant samples

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ISO 13485:2016 ISO 9001:2015

Total RNA Kit

(Spin Column)

Cat. No. ODP419

Kit Contents

Contents	50 Preps
Buffer RZ	50mL
Buffer RD	13mL
Buffer RW	15mL
RNase-free Water	10mL
RNase-free spin column CR3 & Collection tube (2mL)	50
Hand book	1

Storage

Buffer RZ

For short term (≤ 1 month): At room temperature, protected from light

For long term (≥ 1 month): At 2-8°C, protected from light

Other reagents: At room temperature

Note: Buffer RZ may be transported at room temperature

Introduction

Total RNA kit uses a new technology based on guanidine thiocyanate/phenol method. It contains a unique Buffer RZ that minimizes the contamination of genomic DNA and protein. Total RNA kit can efficiently extract high pure RNA from blood, cells, tissues and plant samples in one hour. The extracted RNA is ready-to-use in downstream applications such as: PCR, gene chips assay, northern blot, dot blot, poly A screening, *in vitro* transcript and molecular cloning.

Notes for avoiding RNase contamination

- Change gloves regularly.
- Use RNase-free plasticwares or glasswares to avoid cross contamination.
- To remove RNase, the glassware can be roasted at 150°C for 4 hours, while plastic can be dipped in 0.5M NaOH for 10 minutes, washed by RNase-free Water thoroughly and sterilized.
- Use RNase-free Water. (Add DEPC into water in clean glass container to a final concentration of 0.1% (v/v). Incubate overnight and autoclave for 15 minutes to remove any trace of DEPC).

Protocol

Buffer RD and Buffer RW are supplied as a concentrate. Before use, add ethanol (96-100%) as indicated on the bottle to obtain a working solution.

Note: It is advised to reconstitute ONLY required volume of Buffer RD with ethanol (96-100%) as reconstituted Buffer RD will precipitate on long term storage.

1. Homogenizing samples
 - a. Tissues: Add 1mL Buffer RZ for 20-50mg of sample. Homogenize sample using a micro pestle (use tissue homogenizer if required). Usually, the volume of tissue sample should not exceed 10% of Buffer RZ volume.

Note: Add 2mL Buffer RZ to tissue in which RNA can be difficult to extract (liver, spleen, bone, and cartilage)

- b. Adherent cells (do not use more than 1×10^7 cells): Cells grown in a monolayer in cell culture vessels can be either lysed directly in the vessel (up to 10cm diameter) or trypsinized and collected as a pellet prior to lysis. Cells grown in a monolayer in cell culture flasks should always be trypsinized.
 - i) To lyse cells directly: Add 1mL Buffer RZ directly to the cells in the culture dish per 10cm^2 of culture dish surface area. Pipette the lysate up and down several times.
Note: The volume of Buffer RZ should be determined according to the surface area instead of the number of cells. Insufficient volume can result in DNA contamination of isolated RNA.
 - ii) 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 $300 \times g$ for 5 minutes. Completely aspirate the supernatant.
Note: Make sure that the supernatant has been completely removed. Residual medium could lead to incomplete lysis of cells and reduced yield of RNA.
- c. Suspension Cells: Harvest cells by centrifugation and remove culture medium. Add 1mL of Buffer RZ per 5×10^6 - 10^7 cells from animal, plant or yeast, or 1×10^7 cells of bacteria. Do not wash cells before addition of Buffer RZ to avoid increased chance of mRNA degradation. Samples from some yeast and bacteria may need to be homogenized by using a power homogenizer.
- d. For gram positive bacteria, use Lysozyme (50mg/mL) (Cat. No.: ORT401-01, not supplied) for lysis.
 - Add 180 μ L Buffer LZ (not supplied) to the bacterial pellet, followed by 20 μ L Lysozyme.
 - Incubate for at least 30 minutes at 37°C . Add 1mL Buffer RZ to the tube and mix the contents by repeated pipetting.
- e. Fungus:
 - Harvest $1\text{--}5 \times 10^9$ cells by centrifugation and remove culture medium. Add 600 μ L Buffer LY (not supplied), 100 μ L β -Mercaptoethanol and 10 μ L Lyticase 10U/ μ L (Cat. No.: ORT410, not supplied) to the pellet.
Note: The volume of Buffer LY, Lyticase and β -Mercaptoethanol will vary with cell number and type of cells.
 - Incubate the sample at room temperature for 30 minutes. Occasionally mix the content by inverting the tube. Centrifuge the sample at 4,000 rpm for 5 minutes and remove the supernatant. Add 1mL of Buffer RZ to the pellet. Do not wash cells before addition of Buffer RZ to avoid increased chance of mRNA degradation. Samples from some yeast may need to be homogenized by using a power homogenizer.
- f. Blood: Take fresh blood, and add three volumes of Buffer RZ. Mix thoroughly. (0.75mL Buffer RZ for 0.25mL whole blood).
- g. Serum: Add equal volume of Buffer RZ to 200 μ L serum or plasma, vortex 30 seconds to mix thoroughly.
- h. Peripheral Blood Mononuclear Cells (PBMC): Isolate PBMC from 5mL whole blood using Ficoll-Hypaque separation solution and density gradient centrifugation. Wash the cells with PBS and transfer the isolated cells to a 1.5mL microcentrifuge tube. Add 1mL Buffer RZ to the tube and mix the contents by repeated pipetting.
- i. For RNA extraction from plant samples, it is recommended to use 100-150mg per 1mL of Buffer RZ.

2. Incubate homogenized samples at 15-30°C for 5 minutes, to permit complete dissociation of the nucleoprotein complex.
Optional step: Centrifuge the sample at 12,000 rpm (~13,400 ×g) for 10 minutes at 4°C. Transfer the supernatant to a fresh microcentrifuge tube.
Note: When preparing samples with high content of fat, proteins, polysaccharides, or extracellular material (e.g., muscle, fat tissue, or tuberous plant material), an additional centrifugation may be required to remove insoluble material from the samples. RNA remains in the upper aqueous phase after centrifugation. However, when dealing with fat tissue, the upper phase is a lipid layer that should be discarded. Retain the clear aqueous phase part for next step.
3. Add 200µL of chloroform per 1mL Buffer RZ used for homogenization. Cap the tube securely and mix the sample by inverting the tube for 15 seconds. Incubate for 3 minutes at room temperature.
4. Centrifuge the sample for 10 minutes at 12,000 rpm (~13,400 ×g) at 4°C. The mixture separates into a lower pink phenol chloroform phase, an interphase, and a colorless upper aqueous phase. RNA remains exclusively in the aqueous phase. Pipette the aqueous phase out into a new tube.
5. Add the 0.5 volume ethanol (96%-100%) to the aqueous phase. Mix thoroughly (precipitate may appear in this step).
Note: Add 0.5mL ethanol if the initial volume of Buffer RZ is 1mL.
6. Transfer the sample from step 5, including any precipitate that may have formed, to an RNase- free spin column CR3 placed in a 2mL RNase-free collection tube. Close the lid gently, incubate at room temperature for 5-10 minutes and centrifuge at 12,000 rpm (~13,400 ×g) for 30 seconds at 4°C. Discard the flow- through.
Note: Since the capacity of CR3 is 700µL, the loading-centrifugation step must be repeated for processing all the mixture from step 5.
7. Add 500µL Buffer RD to the RNase-free spin column CR3 (Ensure ethanol has been added). Close the lid gently, and centrifuge at 12,000 rpm (~13,400 ×g) for 30 seconds at 4°C. Discard the flow- through.
8. Add 700µL Buffer RW to the RNase-free spin column CR3 (Ensure ethanol has been added). Close the lid gently, and centrifuge at 12,000 rpm (~13,400 ×g) for 30 seconds at 4°C. Discard the flow- through.
9. Repeat step 8.
10. Set the RNase-free spin column CR3 back to the collection tube. Centrifuge at 12,000 rpm (~13,400 ×g) for 2 minutes at 4°C to dry the spin column membrane. Open the lid of the spin column CR3 and incubate the assembly at room temperature (15-25°C) for 2 minutes to dry membrane completely.
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 1.5mL RNase-free microcentrifuge tube. Add 30-100µL RNase-free Water directly to the spin column membrane. Close the lid gently, and incubate at room temperature (15–25°C) for 2 minutes. Centrifuge at 12,000 rpm (~13,400 ×g) for 2 minutes at 4°C to elute the RNA.
Note: The volume of elution buffer should not be less than 30µL, or it may affect recovery efficiency. To obtain higher productivity, add the solution gained from step 11 to the center of membrane again, let the columns stand for 1 minute, and then centrifuge. Extracted RNA should be stored at –80°C.