Cat. # 9112/9113

For Research Use

TaKaRa

RNAiso Blood
(Total RNA Extraction Reagent)

Product Manual

v201812Da

Table of Contents

I. Description

RNAiso Blood is an RNA extraction reagent that can quickly isolate total RNA from liquid samples, including blood and high-water content plant samples. This reagent allows for a simple protocol; after cell lysis in RNAiso Blood solution, chloroform is added to the homogenate, the solution is mixed well and centrifuged to separate the mixture into 3 layers. The aqueous layer on top contains the RNA, the semi-solid middle layer contains DNA, and the bottom organic layer (red) contains proteins, polysaccharides, fatty acids, cell debris, and a small amount of DNA. Total RNA can be recovered from the top layer by performing isopropanol precipitation.

Using RNAiso Blood, total RNA extraction can be completed in approximately 1 hour. Because the resulting total RNA will be almost completely free of DNA and protein, it can be used directly in downstream applications including RT-PCR*, Northern blot analysis, mRNA isolation, and *in vitro* translation reactions.

* When using the RNA for RT-PCR, small amounts of genomic DNA may affect the results. Therefore, treat RNA samples with Recombinant DNase I (RNase-free) (Cat. #2270A) before use, or use PrimeScript™ RT Reagent Kit with gDNA Eraser (Perfect Real Time) (Cat. #RR047A) for reverse transcription prior to real-time PCR.

<Precautions>

RNAiso Blood is a reagent for samples that contain high water content (such as blood and plant homogenates). Because the reagent contains highly-concentrated denaturants, the yield of RNA will decrease when the reagent is used for solid samples such as mammalian tissue. Use RNAiso Plus (Cat. #9108/9109)* when extracting RNA from solid samples.

* Not available in all geographic locations. Check for availability in your area.

II. Components

RNAiso Blood (Cat. # 9112)*	100 ml
RNAiso Blood (Cat. # 9113)*	200 ml

* Contains protein denaturants; avoid contact with skin, clothing, etc.
In the event of contact with the eyes or skin, rinse immediately with a copious amount of water and seek medical attention.

III. Materials Required but not Provided

- Chloroform
- Isopropanol
- 75% Ethanol (prepared using DEPC-treated water)
- RNase-free water
- Microtubes

IV. Storage

4℃
Protect from light

V. Precautions

1. Commercially-available disposable plastic equipment can be considered RNase-free and used in experiments as is. However, equipment such as microcentrifuge tubes and pipette tips should be autoclaved before use. For items such as glass equipment and spatulas, perform dry heat sterilization at 160℃ for at least 2 hours. Equipment that cannot be dry heat sterilized should be treated with 0.1% diethylpyrocarbonate (DEPC) solution for 12 hours and then autoclaved (in order to prevent carboxymethylation of RNA by the DEPC) before use. Designate equipment for RNA experiments from other equipment.
2. Whenever possible, prepare solutions with 0.1% DEPC-treated water and autoclave before use. If reagents cannot be autoclaved, prepare the solution using equipment and water that have been sterilized.
3. Wear disposable plastic gloves and a mask whenever preparing or handling RNA.

VI. Protocol

1. RNAiso Blood Amount

Sample volume*1	Amount of RNAiso Blood
0.01*2 - 0.25 ml of a liquid sample (blood, biological fluid, or fruit homogenate)	3 volumes (For example, for 0.25 ml of liquid sample, use 0.75 ml of RNAiso Blood.)

*1 Pretreatment with Fruit-mate™ for RNA Purification (Cat. #9192) is recommended for plant samples that contain large quantities of polysaccharides.
*2 The use of Dr. GenTLE® Precipitation Carrier (Cat. #9094) during isopropanol precipitation is recommended when extracting RNA from small sample volumes.

2. Sample Treatment

A. Blood Samples
   1) Mix blood with an anticoagulant. Store at 4℃ when RNA extraction will be performed on the same day; store at -80℃ when RNA extractions will be performed later.
   2) Transfer 0.25 ml of the blood sample to a microtube and add 0.75 ml of RNAiso Blood.
   3) Pipette up-and-down to mix and lyse the cells. Vortexing is not recommended.

B. Other Biological Fluids
   1) After collecting the sample, store at 4℃ when RNA extraction will be performed on the same day; store at -80℃ when RNA extractions will be performed later.
   2) Transfer 0.25 ml of the fluid sample to a microtube and add 0.75 ml of RNAiso Blood.
   3) Pipette up-and-down to mix and lyse the cells. Vortexing is not recommended.

C. Plants with High Water Content

1) For frozen tissue, transfer to a mortar and add liquid nitrogen. Grind to fine powder with a pestle while adding liquid nitrogen. Insufficient grinding will affect the quality and yield of RNA recovered. Add 3 volumes of RNAiso Blood and homogenize.

   For fresh tissue samples, add 3 volumes of RNAiso Blood promptly after collection and homogenize completely.

   Note 1: When treating tissue samples with low water content, add RNase-free water to the sample to obtain a final volume of 0.25 ml. Add RNAiso Blood to perform RNA extraction.

   Note 2: When processing tissue samples that are high in polysaccharides, extract the sample with Fruit-mate for RNA Purification (Cat. #9192) according to the product manual. Then add 0.5 ml of RNAiso Blood to 0.5 ml of the extracted sample.

2) Transfer the homogenized sample to a microtube and allow to stand for 5 minutes at room temperature (15 - 30℃).

3. Extraction of Total RNA

1) Add 0.2 volumes of chloroform to the homogenized mixture from A, B, or C of Step 2. Close the lid and vortex the tube well until the solution becomes a milky white color.
2) Allow to stand at room temperature for 5 minutes.
3) Centrifuge at 12,000 *g* for 15 minutes at 4℃. The solution will separate into three layers: the top aqueous layer (containing RNA), a semisolid middle layer (containing mostly of DNA), and a bottom solvent layer.
4) Transfer the top (aqueous) layer to a new centrifuge tube, taking care not to aspirate the middle layer.
5) Add an equal volume of isopropanol, mix well, and allow to stand at room temperature for 10 minutes.
6) Centrifuge at 12,000 *g* for 10 minutes at 4℃.
7) Discard the supernatant and add an equal volume of cold 75% ethanol. Vortex briefly to wash the pellet. Centrifuge at 7,500 *g* for 5 minutes at 4℃ and discard the supernatant.
8) Dry the pellet at room temperature for several minutes and then dissolve in a suitable amount of RNase-free water.

Note: Do not perform centrifuge drying or heat drying, as it may cause the RNA to be difficult to dissolve.

VII. Overview of RNA Extraction

Biological fluid or plant sample
↓ Add 3 volumes RNAiso Blood and mix well
↓ Homogenize as necessary (for plant tissue samples)
Allow to stand at room temperature for 5 minutes
↓
Add 0.2 volumes chloroform relative to the starting volume (sample + RNAiso Blood)
↓ Vortex well
Allow to stand at room temperature for 5 minutes
↓ Centrifuge at 12,000 *g* at 4℃
Transfer the top (aqueous) layer to a new microtube
↓
Add an equal volume of isopropanol relative to the top (aqueous) layer and mix
↓
Allow to stand at room temperature for 10 minutes
↓ Centrifuge at 12,000 *g* for 10 minutes at 4℃
Wash the pellet with 75% ethanol
↓ Centrifuge at 7,500 *g* for 5 minutes at 4℃
Discard the supernatant
↓ Dry the pellet briefly
Dissolve in a suitable amount of RNase-free water

VIII. Determining Purity of RNA

Agarose Gel Electrophoresis

Load 0.2 - 1.0 μg of heat-denatured total RNA on a 1% agarose gel electrophoresis and stain with ethidium bromide. Intact total RNA will have 2 clear ribosomal RNA bands (28S and 18S in eukaryotes) in a ratio of roughly 2 : 1. If weak or smeared rRNA bands are observed, there is a possibility that the RNA is degraded. In addition, the presence of bands with a higher molecular weight than the rRNA bands suggests genomic DNA contamination. In this case, it is recommended that the RNA be treated with DNase I.

Analysis of RNA quality can also be performed using an Agilent 2100 Bioanalyzer.

Absorbance

Measure absorbance at OD_{260} and OD_{280} using TE Buffer as the diluent. The OD_{260}/OD_{280} ratio should be within the range 1.7 - 2.1.

RNA concentration (μg/μl) = (OD_{260} - OD_{320} x Dilution Ratio x 0.04)

IX. Troubleshooting

1. Low yield

   The amount of purified RNA will differ depending on the sample. The table below shows guidelines for the amount of RNA that can be extracted using RNAiso Blood from a 0.25 ml liquid sample or a 50 mg plant sample.

Sample	Sample Amount	Total RNA Extracted
Human whole blood	0.25 ml	1 - 10 μg
Mouse whole blood	0.25 ml	1 - 10 μg
Bovine whole blood	0.25 ml	1 - 10 μg
Carp whole blood	0.25 ml	10 - 100 μg
Spinach leaf	50 mg	30 - 60 μg
Tomato fruit	50 mg	1 - 10 μg
Orange fruit	50 mg	1 - 10 μg

   If the yield is low, consider the following factors:

   a. Inadequate homogenization of the sample after the addition of RNAiso Blood.
   b. Insufficient recovery of the aqueous layer.
   c. Incomplete dissolving of the RNA pellet.
   d. RNase contamination during isopropanol precipitation, wash, or suspension steps.
   e. Loss of RNA during isopropanol precipitation and wash steps.
      - Use Dr. GenTLE Precipitation Carrier (Cat. #9094) as a coprecipitant during isopropanol precipitation.
   f. Tissue samples had low water content.
      - When using tissue samples with low water content, dilute the sample with RNA-free water to a volume of 0.25 ml before adding RNAiso Blood

2. Low OD_{260}/OD_{280} value (<1.65)
   - Dilute RNA with TE Buffer before measuring the optical density. Low ionic strength and pH value may increase the OD_{280} value.
   - After the addition of RNAiso Blood, allow the mixture to stand at room temperature for 5 minutes. This step is important for separation of nuclear protein from nucleic acids.
   - Ensure that the pipette tip does not come into contact with the middle layer when aspirating the supernatant.
   - Dissolve the RNA pellet well.

3. RNA pellet will not dissolve
   - Excessive drying after the 75% ethanol wash will make dissolution difficult. Take care not to perform heat drying or dry for an excessive time.
   - For dissolving, heat the RNA solution to 60℃ for 5 minutes and allow to stand on ice for several hours.

4. RNA is degraded
   - Use a sample that is fresh and recently collected or has been frozen quickly using liquid nitrogen and stored at -80℃.
   - RNase may have been introduced by the equipment used for RNA extraction.

5. DNA present in extracted RNA
   - The sample used may have contained a large quantity of organic solvent (such as ethanol or isopropanol), high-concentration buffer, or an alkaline solvent.
   - Treat with Recombinant DNase I (RNase-free) (Cat. #2270A).

6. Polysaccharides present in the extracted RNA

   Pretreatment with Fruit-mate for RNA Purification (Cat. #9192) is recommended for plant samples containing a high amount of polysaccharides. In addition, use of High-Salt Solution for Precipitation (Plant) (Cat. #9193) during isopropanol precipitation can help eliminate residual polysaccharides.

X. References

1) Chirgwin J, *et al.* Isolation of Biologically Active Ribonucleic Acid from Sources Enriched in Ribonuclease. *Biochemistry.* (1979) **18**: 5294-5299.
2) Wallace D. Large-and Small-Scale Phenol Extractions. *Methods in Enzymology*. (1987) **152**: 33-41.
3) Coombs L M, *et al.* Simultaneous Isolation of DNA, RNA, and Antigenic Protein Exhibiting Kinase Activity from Small Tumor Samples Using Guanidine Isothiocyanate. *Anal Biochem*. (1990) **188**: 338-343.
4) Nicolaides N C and Stoeckert Jr C J. A Simple, Efficient Method for the Separate Isolation of RNA and DNA from the Same Cells. *Biotechniques*. (1990) **8**: 154-156.
5) Feramisco J R, *et al. Molecular Cloning*: 194-195, Cold Spring Harbor Laboratory, Cold Spring Harbor, N. Y.
6) Raha S, *et al*. Simultaneous Isolation of Total Cellular RNA and DNA from Tissue Culture Cells Using Phenol and Lithium Chloride. *Gene Anal Techn*. (1990) **7**: 173-177.

XI. Related Products

RNAiso Plus (Cat. #9108/9109)*
Dr. GenTLE® Precipitation Carrier (Cat. #9094)
Fruit-mate™ for RNA Purification (Cat. #9192)
High-Salt Solution for Precipitation (Plant) (Cat. #9193)
Recombinant DNase I (RNase-free) (Cat. #2270A/B)
PrimeScript™ RT reagent Kit with gDNA Eraser (Perfect Real Time) (Cat. #RR047A/B)

* Not available in all geographic locations. Check for availability in your area.