Cat. # 9108/9109

For Research Use

TaKaRa

# RNAiso Plus
# (Total RNA extraction reagent)

Product Manual

v201904Da

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## I. Description

RNAiso Plus is a total RNA extraction reagent which can isolate RNA easily and rapidly from animal or plant tissues and cultured cells. After homogenizing the tissues or cells in the RNAiso Plus solution, add chloroform to the homogenate solution, mixed well, and then centrifuge to separate the solution into three layers. The top layer will be a clear liquid containing RNA, the middle layer will be a semi-solid containing DNA, and the bottom layer will be a red colored organic solvent containing proteins, polysaccharides, fatty acid, cell debris, and small amount of DNA.
Remove the top liquid layer and pipet into a new tube. **Be careful not to remove any of the middle layer.** Perform an isopropanol precipitation to extract the total RNA. Using the RNAiso Plus, the total RNA extraction process can be done in about one hour. The isolated total RNA is intact and does not contain a small amount of DNA or proteins, thus it can be used for RT-PCR＊, Northern blot analysis, mRNA isolation, and *in vitro* translation reactions.

＊ If being used for RT-PCR, even an extremely small amount of genomic DNA could affect the result, so treat with Recombinant DNase I (RNase-free) (Cat. #2270A) before use.

## II. Components

| | |
|---|---|
| RNAiso Plus (Cat. #9108)＊ | 100 ml |
| RNAiso Plus (Cat. #9109)＊ | 200 ml |

＊ Contains a protein denaturator, so avoid skin contact. If comes in contact with eyes or skin, wash with water immediately and seek for medical suggestion by a doctor.

［Materials required but not provided］

- Chloroform
- Isopropanol
- 75% ethanol (prepared with DEPC-treated water)
- RNase-free water

## III. Storage

4℃
Store in a dark place to retain activity.

## IV. General Instructions for Handling RNA

1. Sterilized disposable plastic equipments are RNase free in general, thus they can be used for this experiment. Use only autoclaved microcentrifuge tubes or tips for micropipette for this experiment. When using glass equipment or spatulas, perform dry heat sterilization at 160℃ for two hours.
   Equipment which cannot do dry heat sterilized should be treated with 0.1% diethylpyrocarbonate (DEPC) solution at 37℃ for twelve hours then autoclaved (prevent RNA carboxymethylation with DEPC).
   Note：Be sure to discriminate the equipment for RNA experiments.

2. Try to make most of the reagents with 0.1% DEPC treated water. The reagents should be autoclaved before use. If there is a reagent that cannot be autoclaved, use sterilized equipment and components to prepare the reagent, and sterile filtered before use.

3. Researcher's bare hands are the biggest cause of the RNase contamination. Be sure to use disposable plastic gloves and masks when handling experiment reagents associated with RNA.

## V. Protocol

1. Required Amount of RNAiso Plus for extraction

| Sample types and amounts | RNAiso Plus Volume (ml) |
|---|---|
| Adherent cells on 10 $cm^2$ petri dish | 1 - 2 |
| 5 x $10^6$ - 1 x $10^7$ of non-adherent cells | 1 |
| 100 $\mu$l of white blood cells | 2 |
| 50 - 100 mg of tissue sample<br>・ Tissue which RNA can be easily extract<br>・ Tissue which RNA can be difficult to extract (liver, spleen, born, and cartilage＊1) | <br>1<br>2 |
| 15 - 30 mg of plant material＊2<br>(containing small amount of polysaccharide and phenol) | 1 |
| 2 - 5 x $10^7$ yeast cells＊3 | 1 |

＊1 For RNA extraction from bone and cartilage, it is recommended to use High-Salt Solution for Precipitation (Plant) (Cat. #9193) in combination with RNAiso Plus.

＊2 For RNA extraction from plant samples that contain large amount of polysaccharides, it is recommended to use Fruit-mate for RNA Purification (Cat. #9192) as a pretreatment reagent in combination with RNAiso Plus.

＊3 For RNA extraction from yeast, use Yeast Processing Reagent (for total RNA preparation) (Cat. #9089) as a pretreatment reagent in combination with RNAiso Plus.

2. Reagents
   A. Adherent cells
      1 ) Aspirate off media and wash with 1X PBS, enough to just cover the plate.
      2 ) Add 1 - 2 ml of RNAiso Plus onto a 10 $cm^2$ petri dish of adherent cells, swirl the reagent around in plate to make sure the surface has been covered with RNAiso.
          Note：If the cells are difficult to remove, use a cell scraper.
      3 ) Collect the cells with a pipette and transfer them to the centrifuge tube. Repeatedly pipette several times until cells are completely resuspended.
      4 ) Leave the samples at room temperature (15 - 30℃) for 5 min, then isolate the RNA from nuclear protein.

   B. Non-adherent cells
      1 ) Collect and pipette the cells and media into a centrifuge tube. Centrifuge the tube at 8,000*g* for 2 min at 4℃. Discard supernatant and be care not to disturb the cell pellet.
      2 ) Add 1 ml of RNAiso Plus for every 5 x $10^6$ cells.
      3 ) Pipette up and down until pellet is completely resuspended.
      4 ) Leave at room temperature (15 - 30℃) for 5 min, isolate the RNA from the nuclear protein.

   C. Animal and plant tissue sample
      1 ) Immediately transfer frozen tissue into mortar, add liquid nitrogen, then crush with pestle to homogenize until powdery. (if it is not homogenized into small particles, it may affect RNA quality or the yield amount.) Add an amount of RNAiso Plus to correspond with the amount of tissue that was homogenized. For a fresh tissue sample, add RNAiso Plus immediately after collecting the tissue, and homogenize completely.
      2 ) Transfer homogenized sample into a centrifuge tube, keep at the room temperature (15 - 30℃) for 5 min.
      3 ) Centrifuge the tube at 12,000*g* for 5 min at 4℃.
      4 ) Collect supernatant and transfer it to a new centrifuge tube (do not collect pellet).

3. Extraction of total RNA
      1 ) To the solution from above, 0.2 ml of chloroform per 1 ml of RNAiso Plus used for homogenization. Cap the centrifuge tube and mix until the solution becomes milky.
      2 ) Keep the solution at room temperature for 5 min.
      3 ) Centrifuge at 12,000*g* for 15 min at 4℃. The solution will separate into three layers; top liquid layer (contains RNA), semisolid middle layer (mostly DNA), and bottom organic solvent layer.
      4 ) Transfer the top liquid layer to new centrifuge tube without touching middle layer.
      5 ) Add 0.5 - 1 ml of isopropanol per 1 ml of RNAiso Plus used for homogenization and mix well. Keep the mixture at room temperature for 10 min.
      6 ) Centrifuge at 12,000*g* for 10 min at 4℃ to precipitate the RNA.

4. Cleaning RNA precipitate
   Carefully remove the supernatant, do not touch the pellet. If some isopropanol remains that is not a problem. Add an amount of 75% cold ethanol that was equivalent to the supernatant. Clean the precipitate by vortexing. Now centrifuge the solution at 7,500*g* for 5 min at 4℃ and discard the supernatant. Be care not to disturb the precipitate.

5. Dissolving RNA
   Dry the precipitate by leaving the tube open for several min. After the precipitate is dry, dissolved it with appropriate amount of RNase-free water.
   Note：Do not centrifuge to dry the precipitate or heat the precipitate to dry; it may cause difficulty with dissolving RNA.

## VI. RNA Extraction Flowchart

Cultured cell, Animal or Plant tissue.
↓ Homogenize by adding appropriate amount of RNAiso Plus.
Keep the homogenate at room temperature for 5 min.
↓ Centrifuge at 12,000*g* for 5 min at 4℃.＊
Transfer the supernatant to new centrifuge tube.＊
↓
Add chloroform of 0.2 volume of RNAiso Plus used.
↓ Vortex vigorously.
Keep at room temperature for 5 min.
↓ Centrifuge at 12,000*g* for 15 min at 4℃.
Transfer the upper layer to a new centrifuge tube.
↓
Add isopropanol of 0.5 - 1.0 volume of RNAiso Plus used.
↓
Keep at room temperature for 10 min.
↓ Centrifuge at 12,000*g* for 10 min at 4℃.
Wash the RNA with equivalent amount of 75% ethanol.
↓ Centrifuge at 7,500*g* for 5 min at 4℃.
Discard supernatant and keep precipitate.
↓ Air dry, do not heat to dry precipitate.
Dissolve with appropriate amount of DEPC-treated water.

＊ Required for tissue sample

## VII. Analysis of RNA Purity

Analysis by agarose gel electrophoresis (1% agarose gel with ethidium bromide)

Electrophoresis was used to analyze 1 - 2 μg of heat-denatured total RNA from the above extraction process. For total RNA that has not been degraded there would be two ribosomal RNA (eukaryotic cell: 28S and 18S) bands in a 2 to 1 ratio, but if the ribosomal RNA band are diffuse, some degradation of the RNA may have occurred. Also, if there is a band whose molecular weight is more than 28S, it is recommended to treat the solution with DNase I, as genomic DNA might be present.

Analysis by absorbance

Calculate the ratio of $OD_{260}$ / $OD_{280}$ by measuring absorbance after dilute the RNA solution using TE buffer. It is better to have the $OD_{260}$ / $OD_{280}$ ratio in the range of 1.7 - 2.1.

Example：
RNA concentration calculation method:
RNA concentration (μg / μl) = ($OD_{260}$ − $OD_{320}$) x dilution ratio x 0.04

## VIII. Troubleshooting

1. Small amount of extracted RNA

The amount will differ by type of starting material used. The table below lists the amount of RNA that can be extracted from 1 g of tissue or 1 x $10^7$ cells by using RNAiso Plus.

| Tissue samples | Sample amount | Amount of total RNA extracted |
|---|---|---|
| Mouse Liver | 1 g | About 4,000 - 5,000 μg |
| Mouse Kidney | 1 g | About 3,000 μg |
| Mouse Skeletal muscle | 1 g | About 1,500 μg |
| Mouse Brain | 1 g | About 1,500 μg |
| HL-60 cultured cell | 1 x $10^7$ cells | About 100 μg |
| Tobacco leaf | 1 g | About 1,000 μg |
| White blood cells | 1 x $10^7$ cells | About 20 - 40 μg |
| Whole blood＊ | 1 ml | 15 - 20 μg |
| Carp Skeletal muscle | 1 g | About 50 μg |

＊ Used 1 ml of RNAiso Plus for 100 μl of whole blood

If the collected amount was less than expected, the following reasons should be considered.

1. Insufficient homogenization of the sample after addition of RNAiso Plus.
2. Not enough of the top layer was removed from the three layers isolating solution.
3. RNA precipitate was not completely dissolved
4. RNase was included in either the isopropanol precipitation or cleaning process.

2. $OD_{260}$ / $OD_{280}$ ratio is low ( < 1.65)
    - Measure absorption after diluting RNA with TE Buffer. If the buffer has low ionic strength or pH value then the $OD_{280}$ value could artificially elevated.
    - When the tissue was homogenized with an insufficient amount of RNAiso Plus, it may cause inadequate protein separation. When this occurs, homogenize RNA solution again to remove protein.
    - Homogenated solution was not left at room temperature for 5 min. This step is important to isolate nuclear protein from nucleic acid.
    - Some of the middle layer was removed when collecting the supernatant.
    - The extracted RNA is not dissolved completely (See next section).

3. Extracted RNA does not dissolve
    - The RNA precipitate can be hard to dissolve if was dried too long after washing with 75% ethanol. Avoid heating or centrifuging the precipitate to dry.
    - Heat at 60℃ for 5 min, and leave on ice for several hours could help dissolve precipitate.

4. Extracted RNA is degraded
    - Tissue used for RNA extraction should be fresh or flash frozen with liquid nitrogen then stored at -80℃.
    - RNase contamination from the sample or equipment used for RNA extraction.
    - Not enough of RNAiso Plus was added to the tissue sample that contained large amount of RNase.

5. DNA was present in extracted RNA
    - Too little amount of RNAiso Plus was used. Add the suggested amount or add an additional amount from usage chart.
    - Tissue sample used could contain large quantities of chemicals like: ethanol, isopropanol, high concentration of buffer, or alkaline solvent.
    - If DNA was found to be in the extracted RNA, it is recommended to treat with Recombinant DNase I (RNase-free) (Cat. #2270A/B)

6. Polysaccharide in the extracted RNA
    - In general, plant or animal muscle tissues contain large quantities of polysaccharide. As it is difficult to remove polysaccharide from extracted RNA, it is recommended to apply extra amount of RNAiso Plus when extracting RNA from such tissues.
    - For RNA extraction from plant samples that contain large amount of polysaccharides, it is recommended to use Fruit-mate for RNA Purification (Cat. #9192) as a pretreatment reagent. By addition of High-Salt Solution for Precipitation (Plant) (Cat. #9193) at isopropanol precipitation in purification steps, it is effective to remove polysaccharides from RNA solution.

## IX. References

1) Chirgwin J, *et al*. Isolation of Biologically Active Ribonucleic Acid from Sources Enriched in Ribonuclease. *Biochemistry*. (1979)**18**(24): 5294-5299.
2) Wallace D. Large-and Small-Scale Phenol Extractions. Methods in Enzymology. (1987)**152**:33-41.
3) Coombs L M, Pigott D, Proctor A, Eydmann M, Denner J, and Knowles M A. 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 C J Jr. 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, NY.
6) Raha S, Merante F, Proteau G, and Reed J K. Simultaneous Isolation of Total Cellular RNA and DNA from Tissue Culture Cells Using Phenol and Lithium Chloride. *Gene Anal Techn*. (1990)**7**: 173-177.

## X. Related Products

High-Salt Solution for Precipitation (Plant) (Cat. #9193)
Fruit-mate® for RNA Purification (Cat. #9192)
Yeast Processing Reagent (for total RNA preparation) (Cat. #9089)
Recombinant DNase I (RNase-free) (Cat. #2270A)