Reagent for RNA Extraction

ISOGEN Ⅱ

Manual (Second edition)

Code No. 311-07361

Code No. 317-07363

NIPPON GENE CO., LTD.

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I Product description

ISOGEN Ⅱ is a reagent for extracting total RNA and small RNA from animal tissues and cultured cells.

This product is a homogeneous liquid containing phenol and guanidine capable of isolating RNA in a single step by interacting with cellular components. Unlike the reagents for conventional methods (ISOGEN and ISOGEN-LS), this product does not require a liquid phase separation using chloroform.

When ISOGEN Ⅱ is added to a sample to dissolve or homogenize the sample, and then water is added, DNA, protein and polysaccharide are precipitated (insolubilized) and can be removed by centrifugation (right). High purity RNA can be isolated from the supernatant by ethanol precipitation, and washing and dissolving the precipitates.

■ Features

- Chloroform is not used for RNA isolation.
- Small RNA can be more efficiently extracted compared to using conventional reagents (ISOGEN and the like).
- High molecular weight RNA (> 200 base) and small RNA (< 200 base) can be fractionated (not fractionating is another method)
- DNA contamination is minimal, and the extracted RNA can be used for RT-PCR and quantitative RT-PCR as it is.
- RNA can be extracted in about one hour.

II Product content

Code No. 311-07361

- ISOGEN Ⅱ 100 ml
- Manual 1

Code No. 317-07363

- ISOGEN Ⅱ 10 ml
- Manual 1

III Storage

Refrigeration

- Use completely within 6 months from the day of purchase
- This product is shipped at room temperature. After delivery of the product, store at 2-10℃ for problem-free use.

IV Precautions

- This product is a reagent for research and cannot be used for other objectives, e.g., as a drug. Also, this product should be handled only by persons having basic knowledge of reagents.
- ISOGEN Ⅱ is deleterious substances not for medical use (phenol formulations), and should be handled with great care.
- When using, wear appropriate protective gear (gloves, goggles etc.).
- Ventilate the area well so as not to inhale vapors.
- If the product enters eye or adheres to skin, wash with large amounts of water for at least 15 min and consult a doctor.
- Handle this product in accordance with the descriptions in the manual.
- We are not responsible for problems if this product is not handled in accordance with the manual.
- The product safety data sheet (MSDS) can be reviewed on our website (URL http: //www.nippongene.com/pages/products/msds.html)

V Protocol

- In addition to this product, obtain RNase free water, ethanol and isopropanol.
- Separately obtain p-Bromoanisole and Glycogen as necessary.
- Transparent polypropylene tubes may be used. Before using, confirm that the tubes are rated for the centrifugal force (12 K × g) and ISOGEN Ⅱ (phenol).
- In the protocol, a method in which 1 ml of ISOGEN Ⅱ is added is described, and in this case 2.0 ml tubes are required. However, the extraction scale may be adjusted to the volume of the centrifuge tube to be used. For example, 1.5 ml tubes can be used by scaling down the protocol to 0.8 (0.8 ml of the supernatant can be recovered by centrifuging a mixture of about 80 mg of tissue + 0.8 ml of ISOGEN Ⅱ + 0.32 ml of RNase free water).
- All the manipulation can be performed at room temperature but it is recommended that centrifuging takes place at 4-28℃.

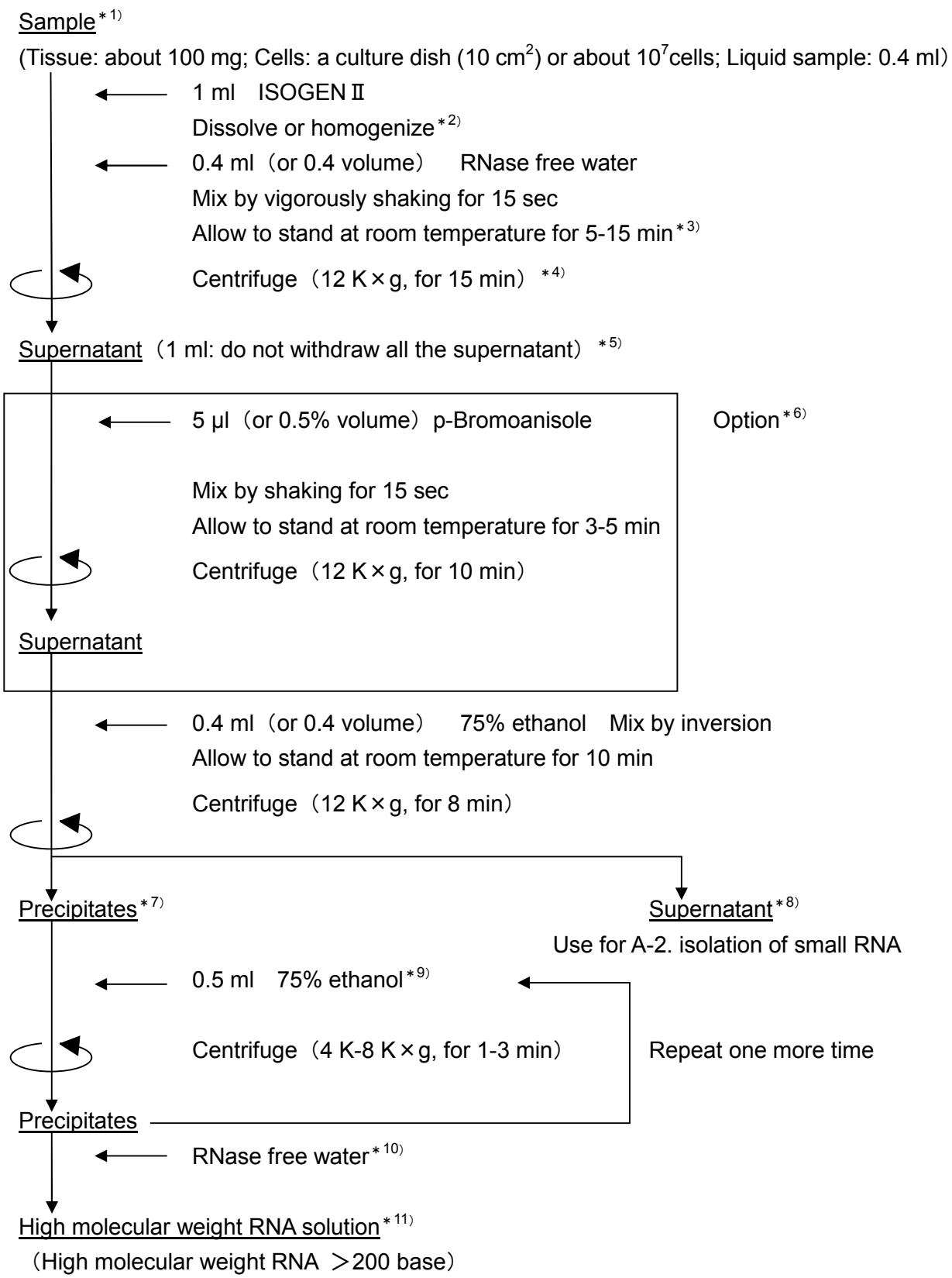
RNA is isolated by the A or B method depending on the sample and the objective.

A: isolation of total RNA
(high molecular weight RNA and small RNA are separately isolated)
A-1: isolation of high molecular weight RNA
B-1: isolation of small RNA

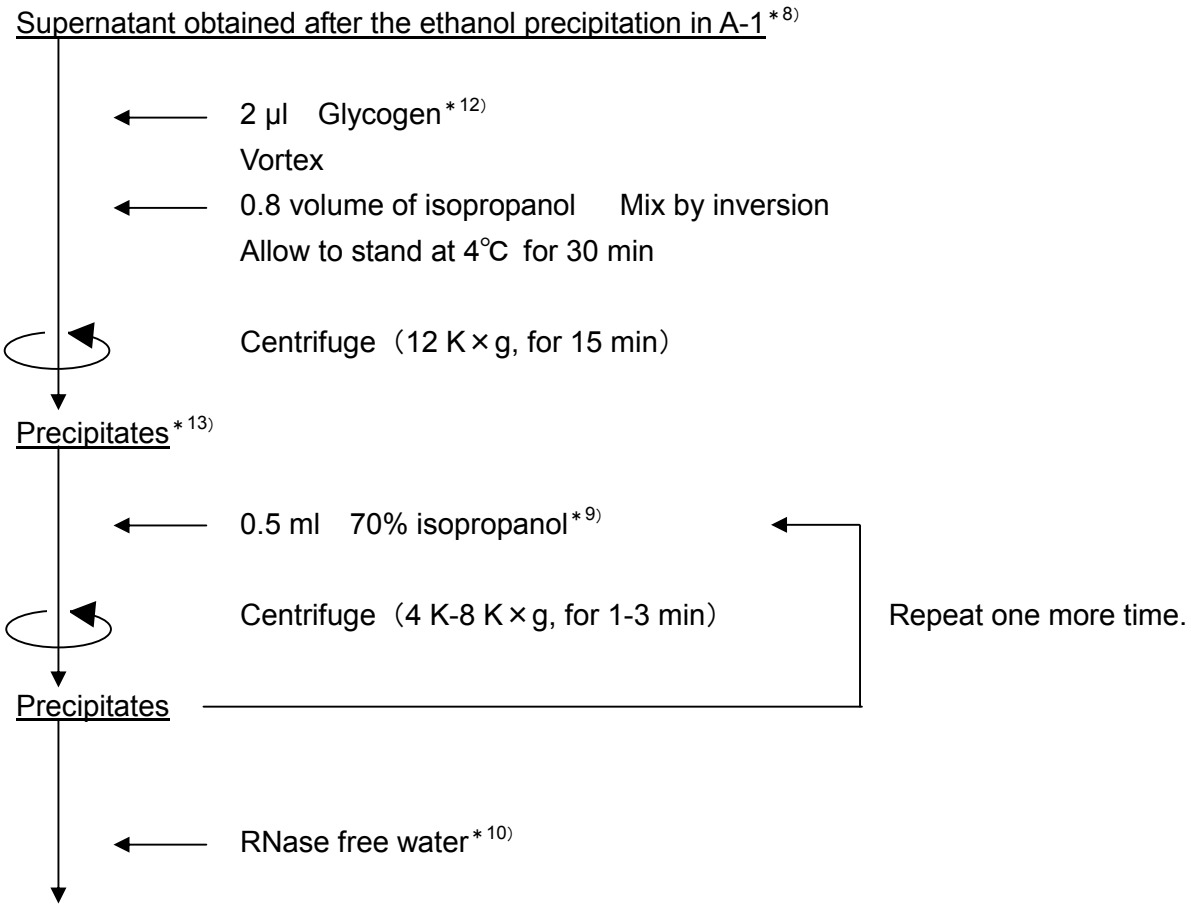
B: isolation of total RNA
(high molecular weight RNA and small RNA are isolated in combination)

A: isolation of total RNA (high molecular weight RNA and small RNA are separately isolated)

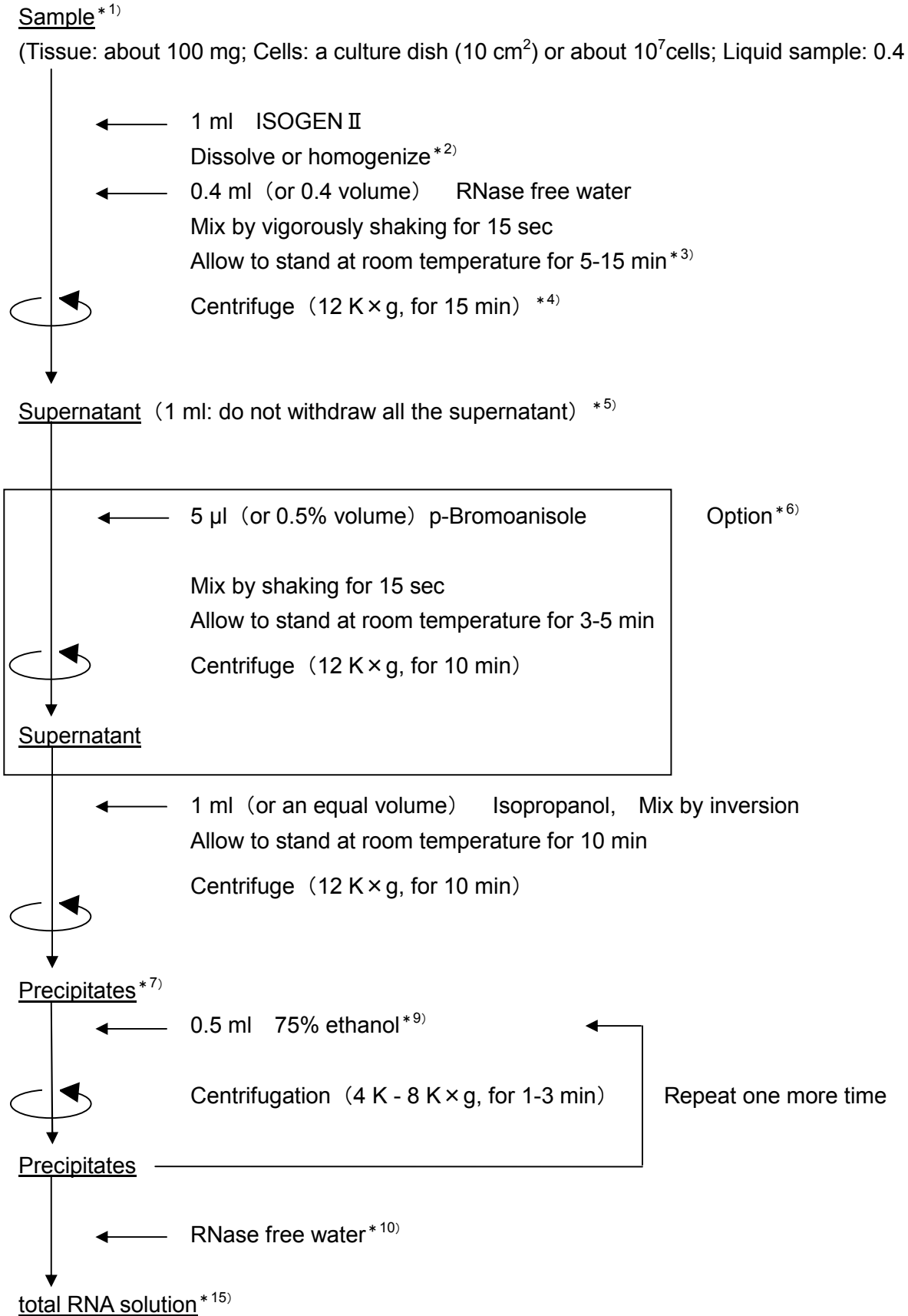
A-1: isolation of high molecular weight RNA



A-2: isolation of small RNA



B: isolation of total RNA（high molecular weight RNA and small RNA are isolated in combination）



*1） **Tissues:** Homogenize up to 100 mg of tissue in 1 ml of ISOGEN Ⅱ using a glass-Teflon homogenizer or polytron homogenizer. In the case of tissues containing many foreign substances (such as the liver and spleen), 50 mg of the tissue should be used per 1 ml of ISOGEN Ⅱ. In the method in the protocol, adding 1 ml of ISOGEN Ⅱ is described, but the scale may be adjusted to the volume of the centrifuge tube to be used. The remaining homogenate can be stored by freezing. See *2).

Attached cells: Remove medium from the culture dish and add at least 1 ml of ISOGEN Ⅱ per 3.5 cm dish (10 cm^2) and completely dissolve by pipetting. The amount of ISOGEN Ⅱ to be used is based on the area of the culture dish, not on the number of cells.

Suspended cells: After precipitating the cells by centrifuge, remove the medium and add at least 1 ml of ISOGEN Ⅱ per 10^7 cells and dissolve cells by pipetting. Since there is possibility of RNA degradation in cultured cells due to pre-treatment such as trypsin treatment and washing, add ISOGEN Ⅱ immediately after the removal of the medium. Also, if the amount of ISOGEN Ⅱ to be used is insufficient to the sample, the isolated RNA may be contaminated by DNA.

Liquid sample: Add 1 ml of ISOGEN Ⅱ up to 0.4 ml of the liquid sample and dissolve. When the sample of less than 0.4 ml is treated, mix the sample with 1 ml of ISOGEN Ⅱ, and then add RNase free water to bring the volume of the mixture to 1.4 ml. Thereafter, go through the process of allowing to stand at room temperature for 5-15 min.

Whole blood: ISOGEN-LS is recommended.

Sample with high lipid content: Centrifuge the homogenate once at 12 K×g for 5 min. Since lipid aggregates on the top layer, collect the supernatant with a pipette or syringe passed through the lipid layer and transfer to a new tube. By performing the centrifugation at 4-10°C, the lipid layer is solidified and easy to handle.

*2） It is important to quickly excise the tissue and to homogenize thoroughly in order to keep the purity and yield of RNA high.

- Immediately homogenize the excised tissue in ISOGEN Ⅱ or freeze in liquid nitrogen.
- The most effective homogenization method is to crush for 2-3 min with a polytron homogenizer set at high speed. Since the brain sample tends to foam, use a glass-Teflon homogenizer.
- If a sample with high RNase content is used, use cooled ISOGEN Ⅱ to prevent RNA degradation.
- To measure tissue, put 1-5 ml of ISOGEN Ⅱ in a tube beforehand and place in an electronic balance to adjust to zero. Put the fresh tissue immediately after excision or the frozen tissue in the tube, measure the weight and homogenize immediately. After

crushing the tissue, add the deficit amount of ISOGEN Ⅱ and re-suspend (for example, adjust the ratio to 80 mg of tissue per 1 ml of ISOGEN Ⅱ).

- The homogenate can be stored at 4°C overnight and at -20°C or -70°C for at least 1 year. The frozen homogenate can be thawed by incubating at 37-40°C for 5 min.

*3) When 100 mg of tissue, i.e., the maximum amount of the sample that can be processed, is treated with 1 ml of ISOGEN Ⅱ or the sample contains a large amount of DNA, allow to stand for 15 min.

*4) After centrifugation, much of the DNA, proteins, polysaccharides and the like will form blue, semisolid precipitates at the bottom of the tube. RNA is solubilized in the supernatant. For example, when 100 mg of tissue is treated with 1 ml of ISOGEN Ⅱ, the volume of the precipitates of DNA and protein is about 10% of the total volume of the mixture of the homogenate and water (if 80 mg of tissue is treated with 1 ml of ISOGEN Ⅱ, the ratio would be about 8%).

*5) Carefully collect 1 ml of the supernatant from the top (75% of the total volume of the supernatant) and transfer to a new tube. At this time the blue color still remains in the supernatant. Since the precipitates contain DNA, be careful not to collect the supernatant near the precipitates in order to prevent DNA contamination.

*6) Since contaminating DNA, proteins, polysaccharides and the like are precipitated by performing this optional manipulation, this method is effective for samples with high impurity content (especially tissue from the liver, kidney, spleen and muscles).

*7) The white precipitates of RNA are attached to the bottom of the tube.

*8) Transfer the supernatant to a new tube and store at 4°C or -20°C to isolate small RNA. At this time, carefully collect 85% of the supernatant from the top so that the contamination by high molecular weight RNA is prevented. The supernatant at this stage retains a pale blue color. This supernatant can be stored at -20°C for at least a year.

*9) In this condition, samples can be stored at room temperature overnight, at 4°C for a week and at -20°C or -70°C for a year.

*10) After removing the supernatant with a micropipette, dissolve the RNA precipitates with an appropriate amount of RNase free water without drying. If the RNA precipitates are dried, the solubility is markedly reduced. The RNA precipitates are preferably dissolved by vortexing or pipetting and allowed to stand at room temperature for 2-5 min. Use RNase-free tubes.

*11） The isolated RNA at this stage is high molecular weight RNA of not less than 200 bases containing rRNA and mRNA. These RNA account for about 80-85% of intracellular RNA. The remaining small RNA fraction can be isolated using protocol A-2.

*12） Small RNA can be efficiently recovered by adding glycogen.

*13） Be aware that the precipitates at this stage are sometimes difficult to see.

*14） RNA isolated here is small RNA of 10-200 base.

*15） RNA isolated here is total RNA containing high molecular weight RNA and small RNA.

Estimated RNA yields obtained using protocols A and B are as follows.

Sample		Protocol A-1	Protocol B
Tissue	Liver	5-7 µg RNA/mg tissue	6-8 µg RNA/mg tissue
	Kidney, Spleen	3-4 µg RNA/mg tissue	3-4 µg RNA/mg tissue
	Skeletal muscles, Brain, Lung	0.5-1.5 µg RNA/mg tissue	0.5-1.5 µg RNA/mg tissue
	Placenta	1-3 µg RNA/mg tissue	1-3 µg RNA/mg tissue
Cultured cells	Epithelial cells	5-8 µg RNA/10^6 cells	5-10 µg RNA/10^6 cells
	Fibroblasts	3-5 µg RNA/10^6 cells	4-6 µg RNA/10^6 cells

A_{260}/A_{280} of isolated RNA is 1.7-2.1. Use a buffer of pH 8.0 or higher such as TE (pH 8.0) to accurately measure absorbance.

VI Troubleshooting

Trouble	Countermeasure
Low yield	Thoroughly homogenize and dissolve the sample. See *2).
	Thoroughly dissolve the obtained RNA precipitates.
$A_{260}/A_{280} < 1.6$	Increase the amount of ISOGEN Ⅱ to be added to the sample.
	Use a buffer of pH 8.0 or higher such as TE (pH 8.0) to measure the absorbance.
	Thoroughly dissolve the obtained RNA precipitates.
	Since there is a possibility of the contamination of proteoglycan and polysaccharide, take the countermeasures below.
RNA degradation	Perform ISOGEN Ⅱ treatment immediately after excising the tissue, or quickly freeze with liquid nitrogen.
	Store the frozen samples at -70°C.
	For cultured cells, do not perform pretreatments such as trypsin treatment or washing.
	Solutions and tubes used for dissolving RNA should be RNase free.
	Perform optional manipulations. See *6).
Contamination by DNA	Increase the amount of ISOGEN Ⅱ to be added to the sample.
	Do not use samples containing organic solvents, strong buffers, salts or alkaline solutions.
	After adding water to the homogenate, allow the homogenate to stand for 15 min and centrifuge at 16 K × g to precipitate DNA.
	Cautiously collect the supernatant after the DNA precipitation. See *5).
Contamination by fat, proteoglycan, and polysaccharide	Remove fat and precipitates (insoluble materials) by centrifuging (12 K × g, for 10 min) the homogenate.
	Perform optional manipulations. See *6).

VII References

1. Chomczynski, P.: Reagents and methods for isolation of purified RNA, US and International Patents Pending.
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4. Wilfinger, W., Mackey, K. and Chomczynski, P.: ”Effect of pH and ionic strength on the spectrophotometric assessment of nucleic acid purity”, Biotechniques, 22, 474-481（1997）

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