Reagent for RNA Extraction

ISOGEN

ISOGEN-LS

Manual Ver. 3rd

NIPPON GENE CO., LTD.

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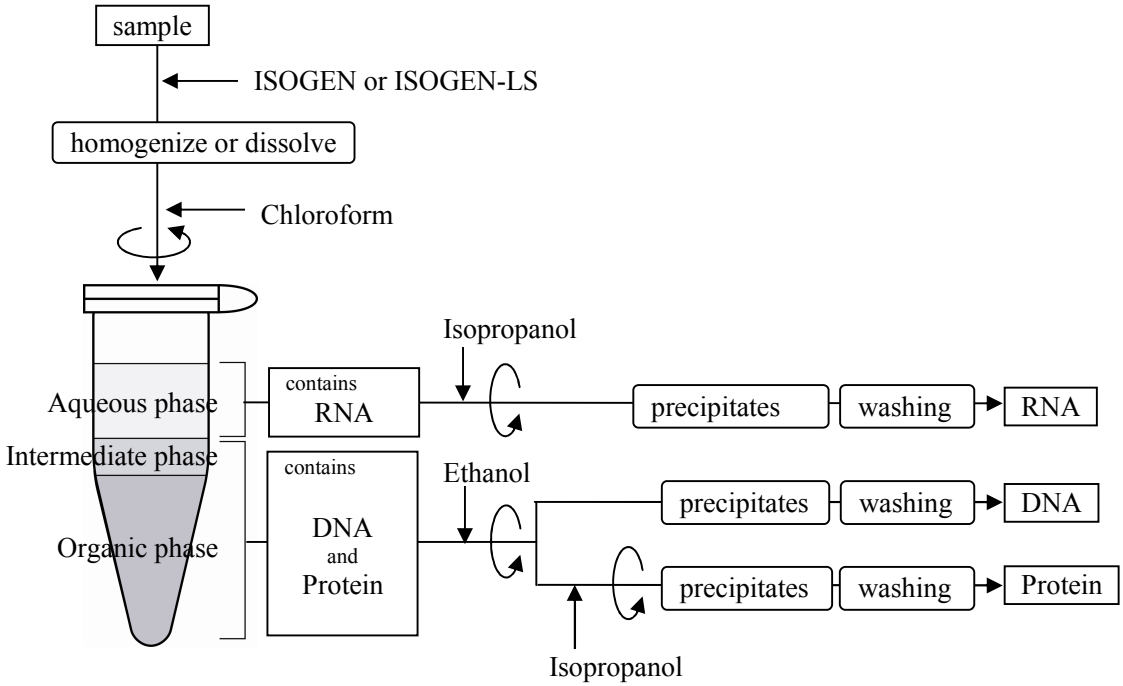
Precautions:

- This product is a reagent for research and cannot be used for other objectives. Also, this product should be handled only by persons having a basic knowledge about reagents.
- ISOGEN and ISOGEN-LS are deleterious substances not for medical use (phenol formulations), and should be handled with great care.
- When using, wear appropriate protective gear (gloves, goggles, etc.).
- 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 caused if this product is not handled in accordance with the manual.
- The product safety data sheet (SDS) can be reviewed on our website (URL http://www.nippongene.com/)

I. Principle

ISOGEN and ISOGEN-LS are reagents for RNA extraction from humans, animals, plants and bacteria. The liquid phase separation method is used, and DNA and protein can be isolated from a single sample. Since RNA, DNA and protein can be isolated through a series of manipulations, it is very effective for analyzing precious samples, and the simplicity of the manipulations allows a large number of samples to be handled.
ISOGEN and ISOGEN-LS are homogenous liquids containing phenol and guanidine thiocyanate, with ISOGEN-LS containing more phenol than ISOGEN. ISOGEN is used for the samples of tissues and cultured cells, and ISOGEN-LS is effectively used for samples of large volume such as blood (> 100 µl when processed in a 1.5 ml plastic tube) and liquid samples that are difficult to concentrate. ISOGEN or ISOGEN-LS is added to a sample, and after dissolving or homogenizing, chloroform is added to the mixture and centrifuged. The homogenate is separated into 3 phases: an aqueous phase, an organic phase and an intermediate phase. In the aqueous phase, only RNA is present, and DNA and protein are present in the intermediate phase and the lower phase. The aqueous phase is collected, and RNA is precipitated by adding isopropanol. DNA is precipitated by adding ethanol to the residual intermediate phase and organic phase, and protein is then precipitated by adding isopropanol. In this way, RNA, DNA and protein can be sequentially isolated.



Storage:
2°C - 10°C

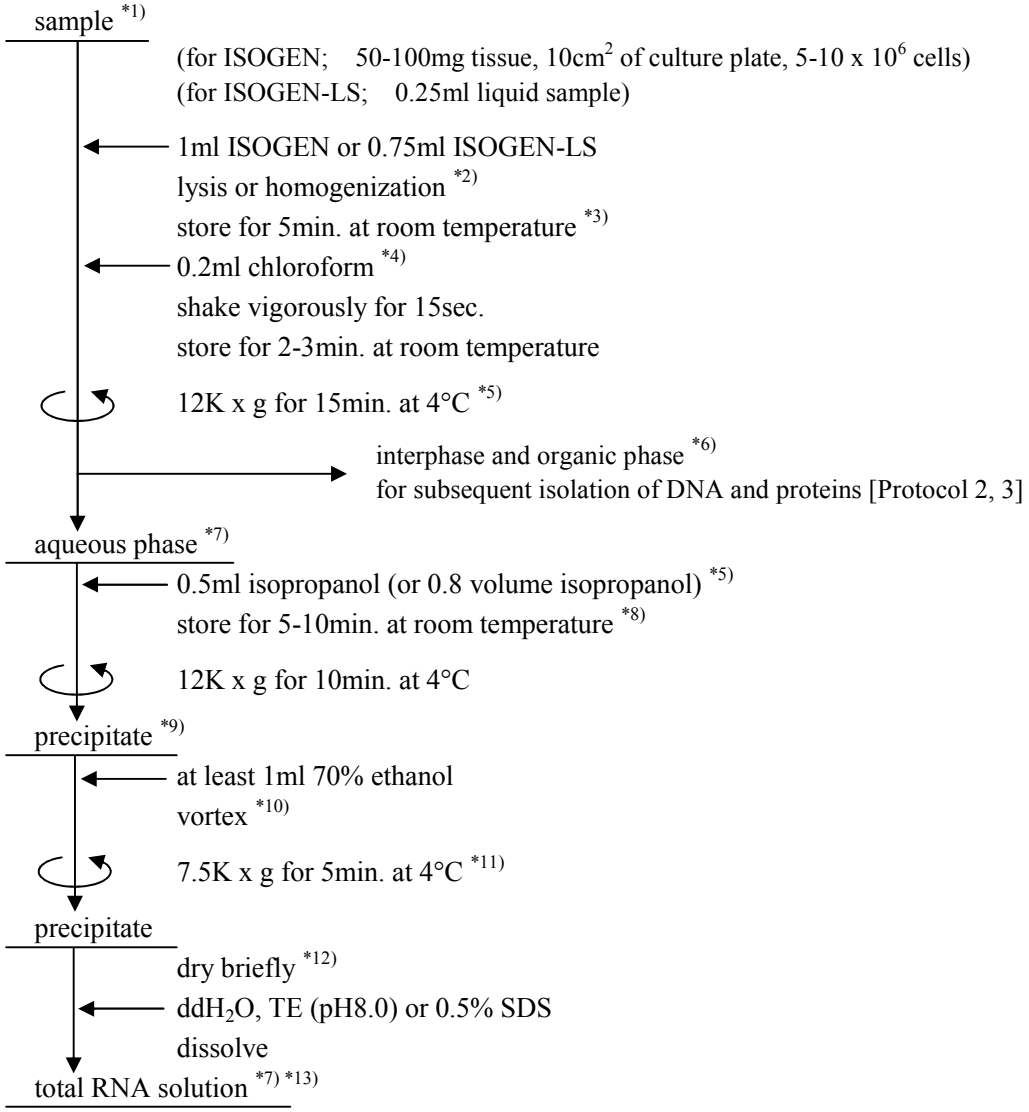
- Use this product as soon as possible after opening the bottle (within 6 months from the purchase date).
- Do not use the product if discoloration in the product is observed.
- Although the product is shipped at room temperature, it can be used safely by storing it at 2-10°C after delivery.

II. RNA isolation

When isolating RNA using ISOGEN or ISOGEN-LS, intact RNA can be isolated in about one hour at a high yield. The RNA thus obtained contains almost no DNA or protein, and can be used as-is for Northern analysis, dot blot hybridization or the like without DNase and other treatments.

[Protocol 1-1, RNA isolation]

The following manipulations should be performed under a clean environment by wearing gloves not only to prevent RNase contamination but also for safety. Further, chloroform, isopropanol and ethanol should be obtained from other sources. Also, tubes made of clear polypropyrene are preferable.



*1) When samples are treated in a 1.5 ml plastic tube, use ISOGEN for samples with volumes of 100 µl or less, such as the tissues and culture cells. ISOGEN-LS is effective for samples with greater volume (over 100 µl) such as the blood. Even if the sample volume is large, ISOGEN may be used if the volume can be reduced to 100 µl or less by concentration. In the case of samples such as cultured cells, remove fluid such as mediums as much as possible.
When treating a liquid sample using ISOGEN-LS, if the sample volume is less than 0.25 ml, add water to bring up the volume to 0.25 ml. Make sure that the volume ratio of ISOGEN-LS to the sample is always 3:1. Liquid samples containing many components such as blood and serum should be diluted two-fold with water, followed by the addition of ISOGEN-LS. Since heparin inhibits reverse transcriptase activity, sometimes RT-PCR and PCR do not work properly using total RNA extracted from blood samples collected using heparin. EDTA does not inhibit reverse transcriptase activity.

*2) The tissues are homogenized with a glass/Teflon or a Polytron homogenizer. Cells grown attached to culture plates are dissolved by directly adding 1 ml of ISOGEN per 10 cm^2(1 well/6-well plate) by pipetting. In the case of cell suspension, cells are precipitated by centrifuge and then dissolved by adding 1 ml of ISOGEN per 5-10x10^6 cells by pipetting. Samples such as muscle, lipocyte and plant, which contain large amount of protein, lipid, sugar and the like, are centrifuged (12K x g, 10 min, 4°C) once after the homogenization, and the supernatant is used for subsequent manipulations. Note that fat is located in the top layer.

*3) In this state, the sample can be stored at -70°C for at least one month.

*4) Chloroform mixed with isoamyl alcohol may not be used.

*5) The homogenate is separated by centrifugation to an organic phase in the lower layer, the intermediate layer and an aqueous phase in the top layer. The aqueous phase contains RNA but almost no DNA or protein. The volume of the aqueous phase is about 60% of the volume of added ISOGEN. If the volume is less than 60%, the volume of isopropanol to be added in the manipulations thereafter should be 0.8 times the volume of the aqueous phase. If the volume is less than 30%, we recommend extracting again after reducing the amount of tissue.

*6) The intermediate phase and organic phase obtained here are stored at 4°C to isolate DNA and protein, respectively. However, if the intermediate phase and organic phase contain a large amount of insoluble material (sullage, fibrous material and the like), they are not suitable for isolation of DNA and protein.

*7) To improve the recovery rate of RNA by reducing contamination by DNA and protein, the aqueous phase is transferred to a new tube before performing the subsequent manipulations so that the mixing of the organic phase and intermediate phase into the aqueous phase is minimized. If contamination of genomic DNA is anticipated, extract again by adding an equal volume of chloroform to the aqueous phase transferred to the new tube. If the presence of contaminants such as polysaccharide, proteoglycan and glycogen is anticipated or confirmed in the final RNA preparation, it would be effective to precipitate only RNA by adding the equivalent of half the volume of the aqueous phase of a high-salt precipitation solution (Code No. 313-06341) and isopropanol and centrifuging at 10K x g for 15 min. Thereafter, the precipitates are washed with

70% ethanol.
If the amount of RNA is 10 μg or less, the RNA yield can be improved by adding 3 μl of Ethachinmate to the homogenate or to the aqueous phase.

*8) In this state, RNA precipitates are normally invisible.

*9) RNA precipitates adhere to the inner wall and the bottom of the tube as gel-like pellets.

*10) In this state, the sample can be stored at least for 1 week at 4°C or for 1 year at -20°C.

*11) If RNA precipitates are about to detach from the inner wall of the tube, centrifugation is performed at 12K x g after ethanol washing.

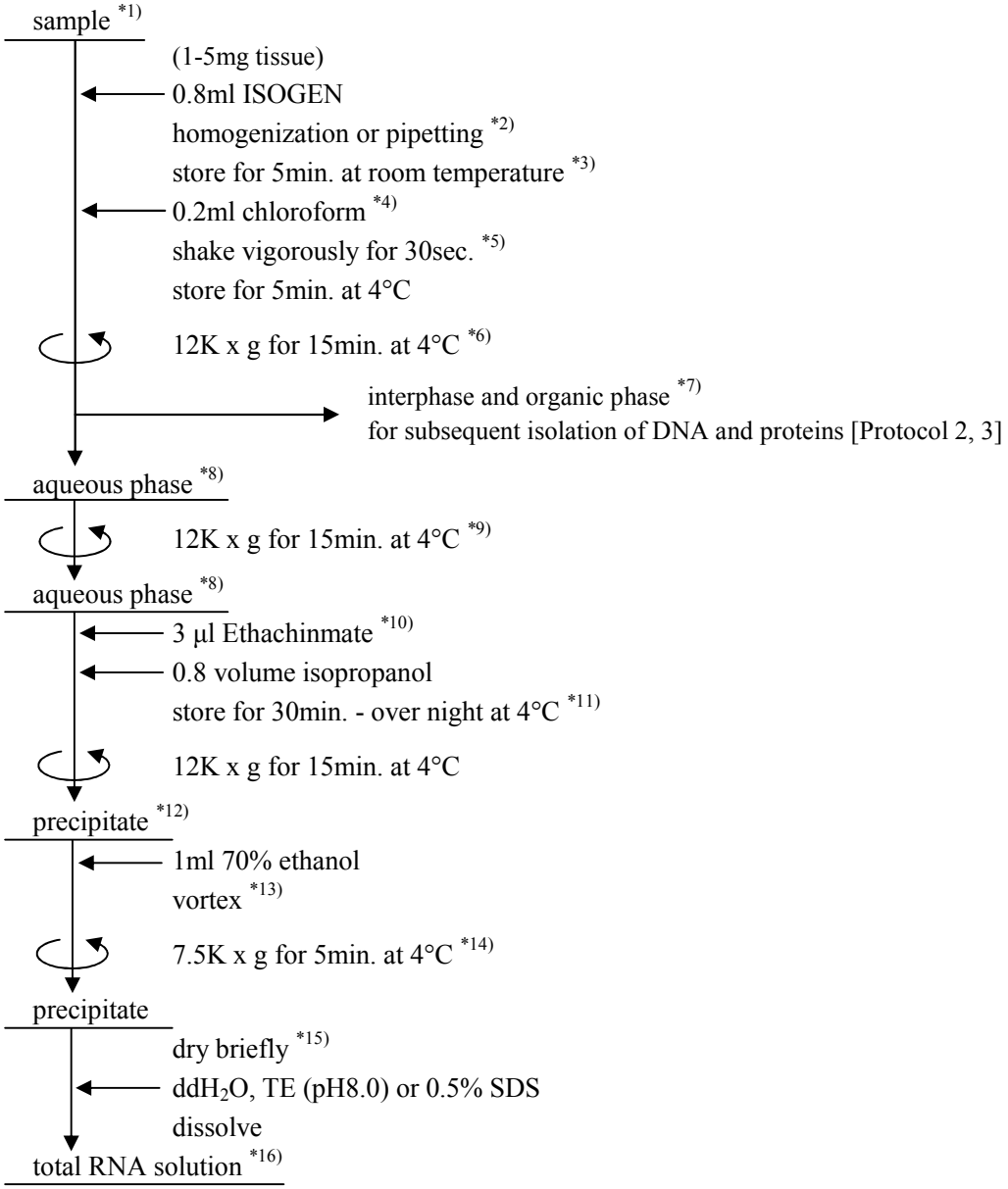
*12) RNA precipitates are air dried or vacuum dried for 5-10 min. Do not dry using a vacuum centrifuge. The solubility is drastically reduced when the precipitates are completely dried. When the solubility of the RNA is reduced, OD_{260}/OD_{280} drops below 1.6, allowing it to be used as a marker. Thus obtained RNA is dissolved in sterilized distilled water, TE (pH 8.0) or 0.5% SDS by sucking with a pipette tip and is preferably incubated at 55-60°C for 10-15 min. Distilled water and 0.5% SDS are made RNase-free by diethylpyrocarbonate (DEPC) treatment beforehand, or commercially available RNase-free products should be used. If TE (pH 8.0) is to be used, a commercial product of RNase-free grade should be used (because Tris-HCl included in TE is degraded by the DEPC treatment).

*13) When RNA obtained is electrophoresed in agarose gel, apart from the main bands of about 2 kb and 5 kb ribosomal RNA, low molecular RNA of 0.1-0.3 kb and high molecular RNA of 7-15 kb can be observed. The OD_{260}/OD_{280} of the RNA obtained is between 1.6 and 1.8. The measurement of OD_{260}/OD_{280} is performed by dissolving in water or a buffer of pH>7.5.

[Protocol 1-2: RNA isolation from a minute amount of a sample]

For a minute amount of a sample obtained by biopsy or the like, a slight modification of [Protocol 1-1] may work better.

The following is an example of using a minute amount of a sample (rat pituitary gland or needle biopsy specimen of a human liver). The manipulations should be performed in a clean environment by wearing gloves not only to prevent RNase contamination but also for safety. Further, chloroform, isopropanol and ethanol should be obtained from other sources. Also, tubes made of clear polypropyrene are preferable.



Results we obtained indicated that the yield of RNA using 1-5 mg of a rat pituitary gland or a needle biopsy specimen of a human liver was 4-8 µg/1-5 mg wet weight.

*1) For soft tissues (such as liver), RNA can be extracted from a minute amount of tissue.

*2) Pulverize the tissue using a 1ml glass/Teflon homogenizer or by suction/blow-out using a pipette tip (1 ml) containing ISOGEN.

*3) In this state, the sample can be stored at least for 1 month at -70°C.

*4) Chloroform mixed with isoamyl alcohol may not be used.

*5) Shake vigorously by hand.

*6) The homogenate is separated by centrifugation to the organic phase in the lower layer, the intermediate layer and the aqueous phase in the top layer. The aqueous phase contains RNA but almost no DNA or protein. The volume of the aqueous phase is about 60% of the volume of added ISOGEN. The volume of isopropanol to be added in the manipulations thereafter should be 0.8 volume of the collected aqueous phase. If the volume of the aqueous phase is less than 30%, we recommend extracting again after reducing the amount of the tissue.

*7) The intermediate phase and organic phase obtained here are stored at 4°C to isolate DNA and protein, respectively.

*8) The aqueous phase is transferred to a new tube before performing the subsequent manipulations so that the mixing of the organic phase and intermediate phase into the aqueous phase is minimized. This is to improve the recovery rate of RNA by reducing the contamination by DNA and protein.

*9) When the supernatant is collected, the recovery rate of the aqueous phase portion can be increased by re-centrifuging to separate the contaminating organic phase.

*10) The RNA yield can be increased by adding Ethachinmate to the aqueous phase, which also allows the precipitation of RNA to be visually confirmed.

*11) Contaminants are increased at -20°C or below, and treatment at 4°C is preferable. In this state, RNA precipitates are normally invisible.

*12) RNA precipitates adhere to the inner wall and the bottom of the tube as gel-like pellets.

*13) In this state, the sample can be stored at least for 1 week at 4°C or for 1 year at -20°C.

*14) If RNA precipitates are about to detach from the inner wall of the tube, centrifugation is performed at 12K x g after ethanol washing

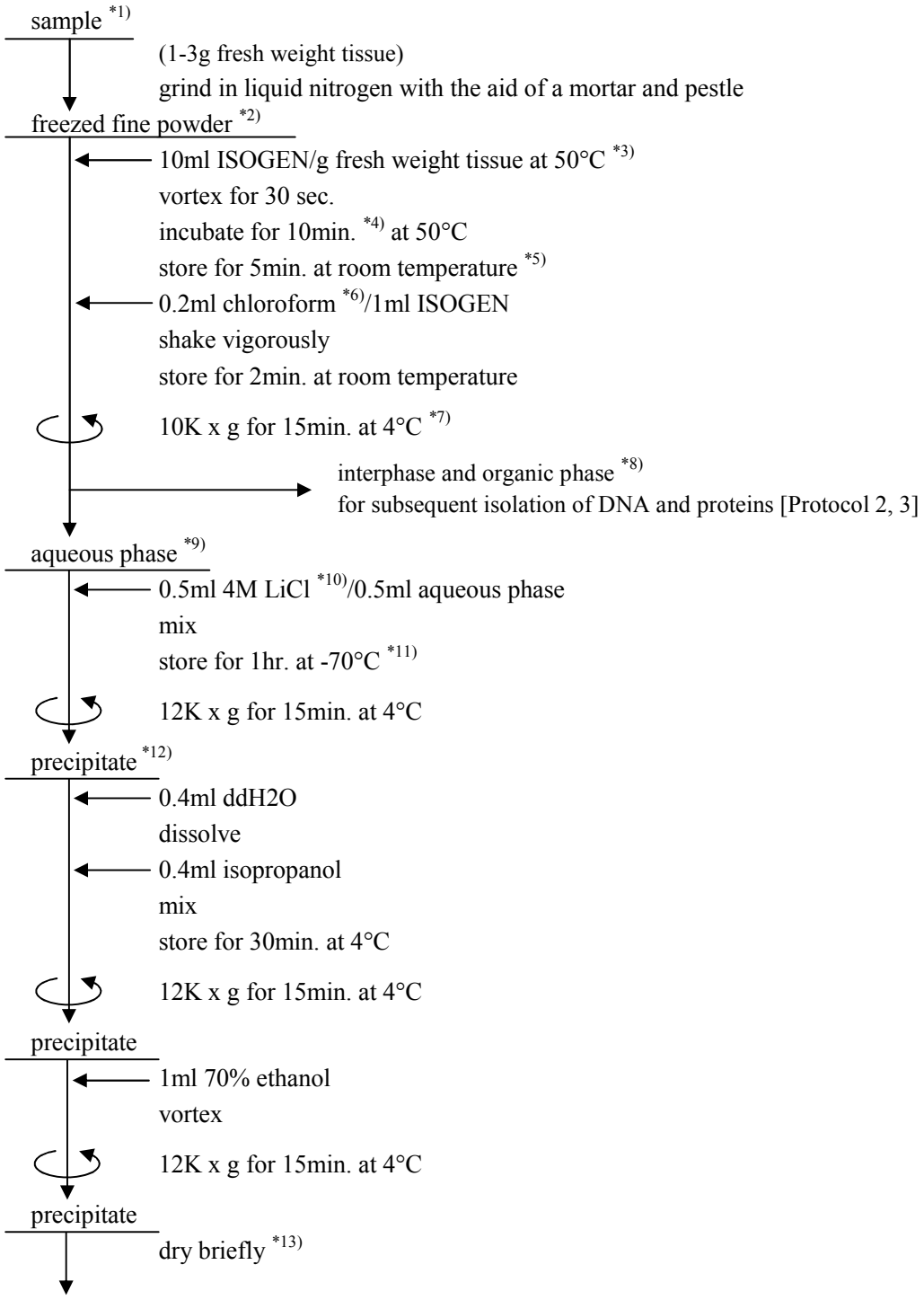
*15) RNA precipitates are air dried or vacuum dried for 5-10 min. Do not dry using a vacuum centrifuge. Solubility is drastically reduced when the precipitates are completely dried. When the solubility of the RNA is reduced, OD_{260}/OD_{280} drops below 1.6, allowing it to be used as a marker. The RNA obtained in this manner is dissolved in sterilized distilled water, TE (pH 8.0) or

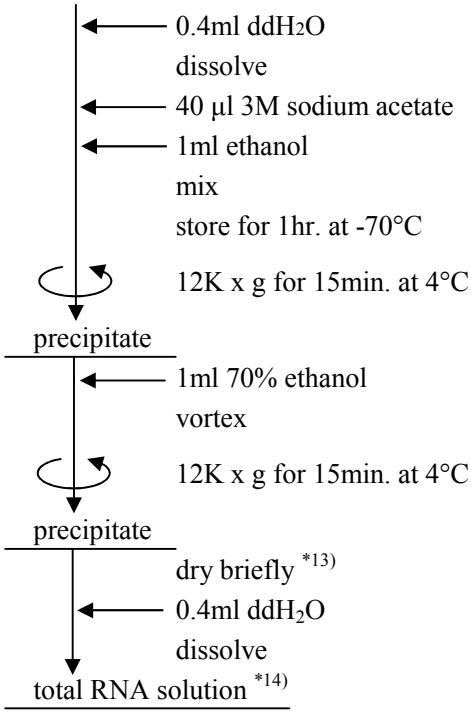
0.5% SDS by sucking with a pipette tip and is preferably incubated at 55-60°C for 10-15 min. Distilled water and 0.5% SDS are made RNase-free by diethylpyrocarbonate (DEPC) treatment beforehand, or commercially available RNase-free products should be used. When TE (pH 8.0) is to be used, a commercial product of RNase-free grade should be used (because Tris-HCl included in TE is degraded by the DEPC treatment).

*16) When RNA obtained is electrophoresed in agarose gel, apart from the main bands of about 2 kb and 5 kb ribosomal RNA, low molecular RNA of 0.1-0.3 kb and high molecular RNA of 7-15 kb can be observed. The OD_{260}/OD_{280} of the RNA obtained is between 1.6 and 1.8. The measurement of OD_{260}/OD_{280} is performed by dissolving in water or a buffer of pH>7.5.

[Protocol 1-3: RNA isolation from plant materials]

The total RNA of a plant sample can be obtained by slightly changing [Protocol 1-1].
The following is an example using a plant sample (*Arabidopsis* or *Tobacco*). The manipulations should be performed under a clean environment by wearing gloves not only to prevent RNase contamination but also for safety. Further, chloroform, isopropanol and ethanol should be obtained from other sources. Also, tubes made of clear polypropyrene are preferable.





The results we obtained indicated that the yield of RNA using plant samples is 150-200 µg ($OD_{260}/OD_{280} \fallingdotseq 1.8$) from 0.5-1 g of fresh weight of *Arabidopsis* or *Tobacco*.

*1) Use samples that are as fresh as possible.

*2) Freeze samples with liquid nitrogen and pulverize, and then keep frozen.

*3) Put ISOGEN (10-20 ml/g fresh weight tissue) into a centrifuge tube beforehand, incubate at 50°C, and put the sample from *2) therein.

*4) Incubate for 10 min or until the sample is completely dissolved. Take note that when the incubation time is too long, there is greater polysaccharide contamination.

*5) In this state the sample can be stored for at least one month at -70°C. If the centrifuge tube used in *3) cannot withstand high centrifugal force (12K x g), subsequent handling will be made easier by distributing 1 ml aliquots of the incubated ISOGEN mixture in which the sample is added, and then by treating the aliquots with chloroform.

*6) Chloroform mixed with isoamyl alcohol may not be used. The amount of chloroform to be added is 1/5 of the volume of ISOGEN.

*7) The homogenate is separated by centrifugation to the organic phase in the lower layer, the intermediate layer and the aqueous phase in the top layer. The aqueous phase contains RNA but almost no DNA or protein. The volume of the aqueous phase is about 60% of the volume of added ISOGEN.

*8) The intermediate phase and organic phase obtained here are stored at 4°C overnight or -70°C for several months to isolate DNA and protein, respectively. However, if the intermediate phase and organic phase contain a large amount of insoluble material (sullage, fibrous material and the like), they are not suitable for isolating DNA and protein.

*9) The aqueous phase is transferred to a new tube before performing the subsequent manipulations so that the mixing of the organic phase and intermediate phase into the aqueous phase is minimized. This is to improve the recovery rate of RNA by reducing the contamination by DNA and protein. 500 µl of the aqueous phase is put in a tube.

*10) White cloud is formed when lithium chloride is added to the aqueous phase.

*11) When liquid nitrogen is used, leave standing for 15 min.

*12) Jelly-like colorless clear soft precipitates and white precipitates are formed. Remove the jelly-like precipitates as much as possible.

*13) RNA precipitates are air dried or vacuum dried for 5-10 min. Do not dry using a vacuum centrifuge. If the precipitates are completely dried, the solubility will be drastically reduced. When the solubility of the RNA is reduced, OD_{260}/OD_{280} is below 1.6, allowing it to be used as a marker.

*14) If the obtained total RNA solution will not be used immediately, store at -80°C by adding 1/10 volume of 3M sodium acetate and 2.5 volume of ethanol. At the time of use, centrifuge at 10K x g for 15 min, wash the obtained precipitates with 70% ethanol, dry and dissolve in sterilized distilled water.
When OD_{260}/OD_{280} is to be measured, dissolve in water or a buffer of pH>7.5.

Estimated yields are as follows:

Sample		Yield
tissue	liver	6-10 µg RNA/mg tissue
	spleen	6-10 µg RNA/mg tissue
	kidney	3-4 µg RNA/mg tissue
	skeletal muscle	1-1.5 µg RNA/mg tissue
	brain	1-1.5 µg RNA/mg tissue
	placenta	1-4 µg RNA/mg tissue
minute tissue	rat pituitary gland or human liver needle biopsy material	4-8 µg RNA/1-5mg tissue
plant tissue	*Arabidopsis* or *Tobacco*	150-200 µg RNA/0.5-1g fresh weight tissue
cultured cells	epithelial cells	8-15 µg RNA/10^6 cells
	fibroblasts	5-7 µg RNA/10^6 cells
blood	(human or animal)	7-15 µg RNA/ml blood

Troubleshooting:

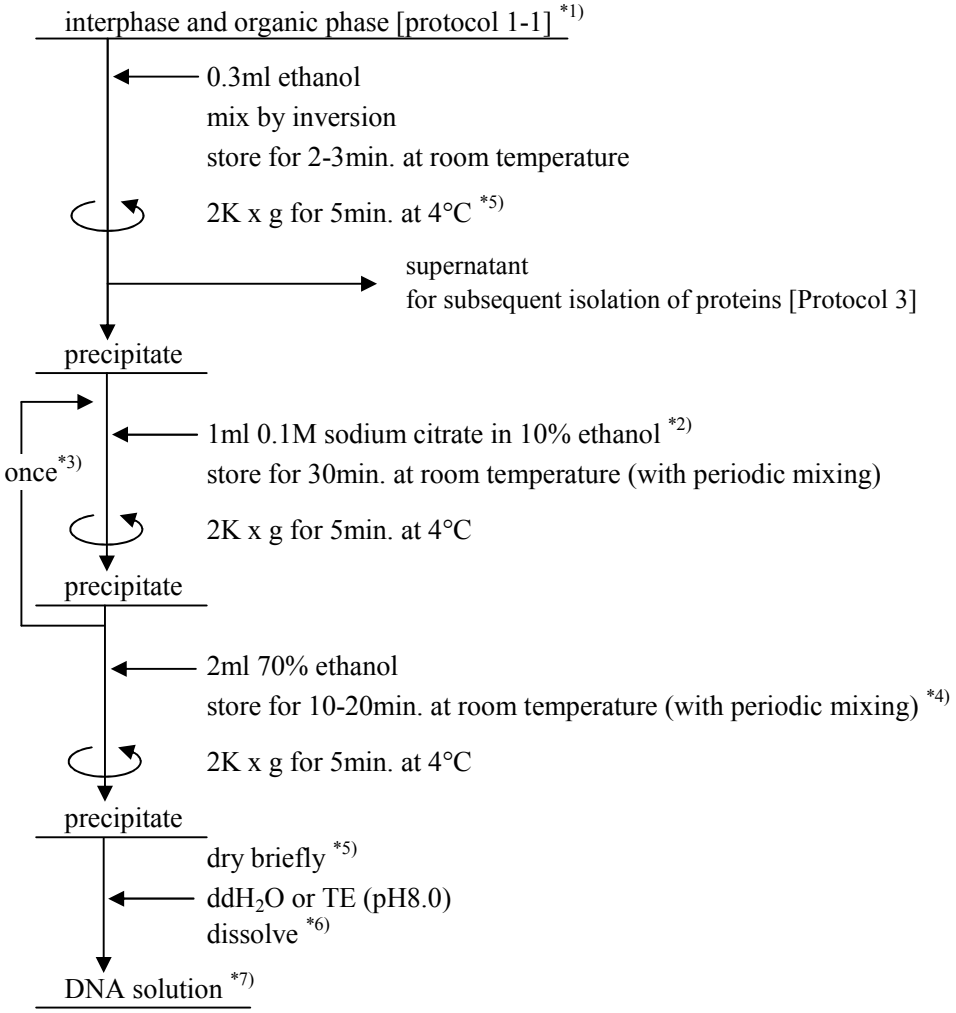
Problem	Counter measure
Low yield	• Dissolve (or homogenize) thoroughly after adding ISOGEN or ISOGEN-LS. • Dissolve obtained RNA precipitates thoroughly.
OD260/OD280<1.65	• Increase the amount of ISOGEN or ISOGEN-LS to be added to the sample. In this case, increase the amount of additives in the subsequent manipulations in proportion to the added ISOGEN or ISOGEN-LS. • After dissolving (or homogenizing) ISOGEN or ISOGEN-LS, immediately add chloroform without leaving at room temperature for 5 min. • When collecting the aqueous phase, make sure that the phenol phase does not mix with the aqueous phase. • Dissolve the obtained RNA precipitates thoroughly. • Before adding chloroform to the sample, centrifuge at 12K x g for 5 min to remove precipitates, and then add chloroform. • Reproducible values can be obtained by dissolving in water or a buffer of pH>7.5.
Degradation of RNA	• Use a fresh sample. • In the case of cells, disperse thoroughly with trypsin digestion. • Perform thorough heat treatment for the solution and containers being used. • In agarose gel electrophoresis, confirm that pH of formaldehyde being used is neutral (degradation occurs at or below pH 3.5).
DNA contamination	• Increase the amount of ISOGEN or ISOGEN-LS to be added to the sample. In this case, increase the amount of additives in the subsequent manipulations in proportion to ISOGEN or ISOGEN-LS. • If DNA contamination is found in the obtained RNA solution, add 1 ml of ISOGEN or 0.75 ml of ISOGEN-LS to this solution (100 µl or less) and perform the treatment again. • Do not use samples containing organic solvents such as ethanol and DMSO, strong buffer solutions or alkaline solutions.

III. DNA Isolation

DNA is recovered from the intermediate phase and organic phase obtained by centrifugation after the homogenization in [Protocol 1-1, 1-2, 1-3]. The isolated DNA can be used as a substrate for PCR and restriction enzymes.
However, if the intermediate phase and organic phase obtained in [protocol 1-1, 1-2, 1-3] contain a large amount of insoluble material (sullage, fibrous material and the like), they are not suitable for isolating DNA because the yield may be small and the purity may be low.

[Protocol 2: DNA isolation]

The following manipulations should be performed while wearing gloves for safety. Further, ethanol and sodium citrate should be obtained from other sources. [Protocol 2] corresponds to [Protocol 1-1] when 1 ml of ISOGEN is used.



*1) To obtain high-purity DNA, the remaining aqueous phase must be carefully and completely removed. The intermediate phase and the organic phase can be kept overnight at 4°C.

*2) [Preparation method of 0.1 M sodium citrate in ethanol]
Add sterilized distilled water to 10 ml of 1M sodium citrate and 10 ml of ethanol to bring up the volume to 100 ml and mix.

[Preparation method of 1M sodium citrate]
Dissolve 29.4 g of tri-sodium citrate 2 hydrate in water, bring up the volume to 100 ml and sterilize by autoclaving at 121°C for 20 min.

*3) Do not reduce washing time. Wash longer if there is more than 200 μg DNA or very large amounts of components other than DNA.

*4) The samples in this state can be stored for several months at 4°C.

*5) Dry in vacuum for 5-10 min. When completely dried, DNA is very difficult to dissolve.

*6) Dissolve by gently sucking with a pipette. At this stage, especially when DNA is isolated from tissue, the sample may still contain an insoluble gel-like substance. In such cases, the gel-like substance is removed by centrifugation (12K x g, 10 min). To further dissolve the insoluble substance, addition of NaOH to a final concentration of 8 mM may be effective. The sample can be stored overnight at 4°C in this state. For longer storage, the sample can be dissolved in TE (pH 8.0) and stored as-is or the sample can be dissolved in sterilized distilled water and adjusted to final concentrations of 10 mM Tris-HCl (pH 8.0) and 1 mM EDTA. When dissolved in NaOH, pH is adjusted to 7-8 by HEPES by referring the table below, and EDTA is added to a final concentration of 1 mM.

*7) At this time, insoluble precipitates are found. To further increase the purity, a phenol / chloroform treatment may be performed. However, in this case the yield may be decreased. When DNA is isolated in accordance with [Protocol 2], 60-100 kb DNA accounts for about 70% and about 20 kb DNA accounts for about 30% of total DNA isolated from cells, and 60-100 kb DNA accounts for about 80% and about 20 kb DNA accounts for about 20% of total DNA isolated from cultured cells. However, since the average length of DNA depends on shearing force during homogenization, the use of a strong homogenizer such as a Polytron homogenizer should be avoided to obtain larger DNA fragments. For the obtained DNA solution, the yield is calculated by measuring OD_{260} after diluting with water. 1 OD_{260} = 50 μg dsDNA/ml. Rough estimation of the yields for 10^6 cells of human, rat and mouse are 7.1 μg, 6.5 μg and 5.8 μg, respectively. The DNA obtained in this manner contains almost no RNA or protein, and OD_{260}/OD_{280} is 1.7 or higher. When used as a substrate of PCR and restriction enzymes, DNA dissolved in sterilized distilled water or TE (pH 8.0) can be used as-is, but for DNA dissolved in NaOH at a final concentration of 8 mM, it is necessary to adjust pH beforehand by HEPES addition or dialysis. The table below may be referred to for adjusting the pH by HEPES. 80-90% of the DNA obtained by this method can be cleaved by 3-5 units of restriction enzyme per 1 μg for 3-24 hours.

OD_{260}/OD_{280} is measured by dissolving DNA in water or a buffer of pH>7.5.

final pH	required HEPES concentration (M)	required HEPES volume (μl/ml 8 mM NaOH)
8.4	0.1	86
8.2	0.1	93
8.0	0.1	101
7.8	0.1	117
7.5	0.1	159
7.2	1	23
7.0	1	32

The estimation of the yield is as follows.

sample		yield
tissues	liver	3-4 μg DNA/mg tissue
	kidney	3-4 μg DNA/mg tissue
	skeletal muscles	2-3 μg DNA/mg tissue
	brain	2-3 μg DNA/mg tissue
	placenta	2-3 μg DNA/mg tissue
cultured cells (human, rat and mouse)		5-7 μg DNA/10^6 cells

Troubleshooting:

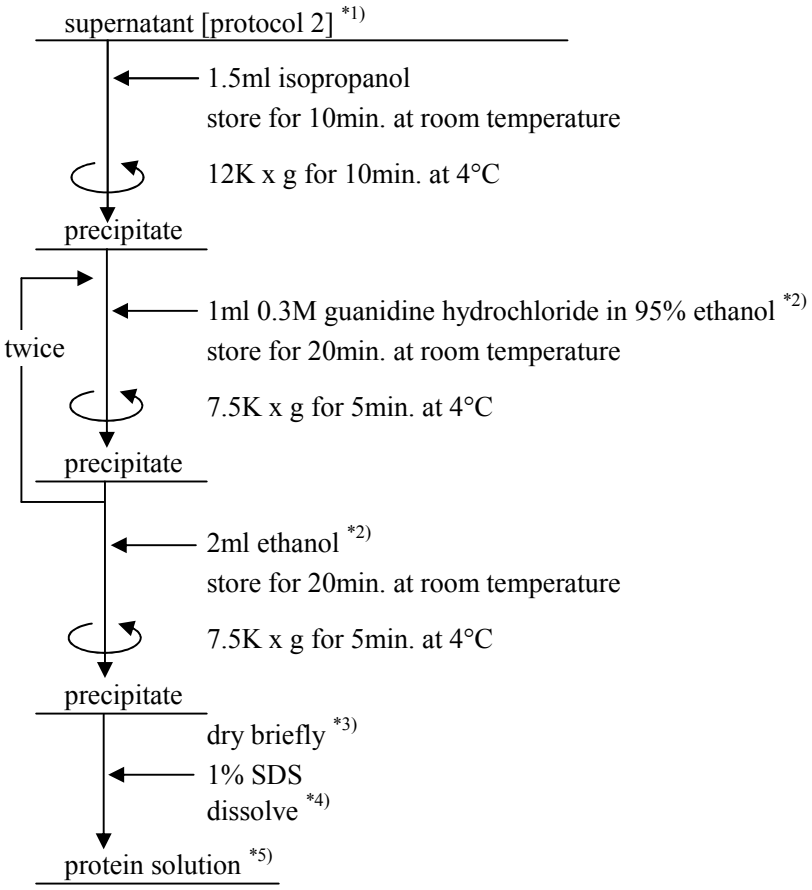
Problem	Counter measure
Low yield	• Thoroughly dissolve (or homogenize) after adding ISOGEN or ISOGEN-LS. • Thoroughly dissolve obtained DNA precipitates. • If insoluble precipitates are found in the obtained DNA solution, disrupt the insoluble precipitates with a pipette tip or the like, centrifuge after incubating at 55°C for 20 min, and then use the supernatant.
OD260/OD280<1.70	• Completely remove phenol by repeating the DNA wash with 0.1M sodium citrate in 10% ethanol. Also, DNA solution loosened by pipetting is centrifuged and the supernatant can be treated with chloroform. However, in this case the recovery rate may be lower. • Reproducible values can be obtained by dissolving in water or a buffer of pH>7.5.
Degradation of DNA	• Use a fresh sample. • Avoid homogenizing samples using a high speed homogenizer such as a Polytron.
Contamination by RNA	• Completely remove the aqueous phase when RNA is isolated. • Thoroughly wash DNA with 0.1 M sodium citrate in 10% ethanol.
Formation of Insoluble precipitates	• Insoluble precipitates may be found when extracted DNA is suspended in sterilized distilled water or TE (pH 8.0). In such cases, centrifuge and use the supernatant. If the yield of DNA obtained from the supernatant is low, sodium hydroxide may be added at a final concentration of 8 mM. Suspending by adding sodium hydroxide may facilitate the solubilization of the insoluble precipitates. To further increase purity, a phenol/chloroform treatment can be performed, but the yield may be reduced.

IV. Protein isolation

Protein is recovered from the organic phase of the supernatant obtained by the centrifugation of the ethanol precipitates from [Protocol 2]. The isolated protein can be used as-is for Western blotting.

[Protocol 3: Protein isolation]

The following manipulations should be performed while wearing gloves for safety. Guanidine hydrochloride, SDS, ethanol and isopropanol should be obtained from other sources. [Protocol 3] corresponds to [Protocol 1-1] when 1 ml of ISOGEN or 0.75 ml of IOSGEN-LS is used.



*1) In this protocol, the amount of the supernatant is about 0.8 ml. The yield may be increased by dialyzing against 0.1% SDS at 4°C.

*2) In this state, the sample can be stored at least for 1 month at 4°C or for at least one year at -20°C.

*3) Vacuum dry for 5-10 min.

*4) The following 3 methods can be used for effectively recovering protein.

A) Dissolve pellets in 1% SDS by pipetting and incubate at 50°C. Since the pellets contains insoluble material, remove them by centrifugation (10K x g, 10 min, at 4°C).

B) Dissolve pellets in 10M urea/50 mM DTT by disrupting with a needle (use a fresh urea/DTT solution). Store the solution at room temperature for 1 hour and heat in a boiling water bath for 3 min. Cool down in ice and sonicate. If not dissolved, perform boiling and sonication twice more until pellets are dissolved.

C) Dialyze the supernatant of phenol/ethanol from *1) against 0.1% SDS at 4°C.

*5) Insoluble materials should be removed by centrifugation (10K x g, 10 min, at 4°C). The protein thus obtained can be used as-is for Western blotting and can be stored at -20°C.

Troubleshooting:

Problem	Counter measure
Low yield	• Thoroughly dissolve (or homogenize) after adding ISOGEN or ISOGEN-LS. • Thoroughly dissolve obtained protein precipitates.
Degradation of protein	• Use a fresh sample.
Band deformation in PAGE	• Thoroughly wash protein precipitates.

V. References

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The information in the descriptions of the products may be changed without prior notification.

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