DNA Extraction Reagent

ISOGENOME

Manual Ver. 2

Code No. 314-08113 (100 ml)

NIPPON GENE CO., LTD.

I Product Description

ISOGENOME is reagent for the isolation of genomic DNA from tissues or cells. The ISOGENOME procedure is based on the use of a guanidine-detergent lysing solution which permits selective precipitation of DNA from a cell lysate. The procedure can be completed in 30 min with DNA recovery of 70-100%. The ISOGENOME protocol is fast and permits isolation of genomic DNA from a large number of samples of small or large volumes. Also, ISOGENOME can be isolated of DNA and RNA from same sample by the use of combination with RNA extraction regent ISOGEN II (Code No. 311-07361).

The isolated DNA can be used without additional purification for applications such as Southern analysis, molecular cloning, and polymerase chain reaction (PCR).

II Components

Code No. 314-08113

- ISOGENOME 100 ml

III Storage

Store at room temperature.

IV 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.
- ISOGENOME is include irritant, and should be handled with great care. When using, wear appropriate protective gear (gloves, goggles, etc.).
- 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.

V Protocol

Materials Required but not Provided

- 99.5-100% Ethanol
- 75% Ethanol
- 8 mM NaOH (use within 1 month of preparation. Alternatively, dilute a stock solution of 2-4 M NaOH solution that has been prepared within the past 6 months to a final concentration of 8 mM NaOH)
- 1M HEPES solution (for neutralization)

Unless stated otherwise, the procedure is carried out at room temperature.

＜Protocol A: Extraction of Genomic DNA＞

Step 1. Lysis of Sample (Homogenization)

1. Add 1 ml of ISOGENOME to Sample (25-50 mg tissue, or up to 1×10^7 cells, either in pellet or in suspension (volume < 0.1 ml)).

＜Quantity of cell or tissue that can be treated with 1 ml of ISOGENOME＞

Sample		Sample Volume	Homogenization
tissue	liver, kidney, lungs, heart, skeletal muscle	25-50 mg	2-a)
	spleen, brain (soft tissues)	5-10 mg	
cell	adherent cell	10 cm^2	2-b)
	cell pellet	1×10^7 cells (< 0.1 ml)	2-c)
	suspended cell	0.1 ml	

2. Homogenize by gently and thoroughly.
   a) For animal tissue, homogenize tissue samples in a hand-held glass/Teflon® homogenizer. Use a loosely fitting homogenizer, with a tolerance of 0.1-0.15 mm or higher. Homogenize 25-50 mg tissue in 1 ml of ISOGENOME by applying as few strokes as possible. Typically, 5-10 strokes are required for complete homogenization.
   For small amounts (5-10 mg) of soft tissues, such as spleen or brain, can be dispersed into smaller fragments and lysed by repetitive pipetting with a micropipette.
   b) For adherent cells, after removing the medium from the dish, add 1 ml of ISOGENOME per 10 cm^2 (equivalent to 3.5 cm dish) directly and lyse by pipetting several times with a micropipette.

c) For suspended cells (cell pellet or cell suspension), after centrifugation to remove the medium, add 1 ml of ISOGENOME to 1 x 10^7 cells (volume <0.1 ml) and lyse cells by repeated pipetting.

3. In the case of animal tissues, store the homogenate for 5-10 min at room temperature. (Homogenate of Step 1 can be stored for 1 month at room temperature or for 10 months at 4°C or -20°C.)

Step 2. Centrifugation (optional) *2

1. In the case of tissues, sediment the homogenate for 10 min at 10,000 x g at 4°C or room temperature.
2. Following centrifugation, transfer the resulting viscous supernatant to a fresh tube. Take care to avoid transferring any insoluble material.

*2 This step removes insoluble tissue fragments, RNA, and excess polysaccharides from the lysate/homogenate. It is required for the isolation of DNA from tissues such as liver, muscles containing a large amount of cellular and/or extracellular material. This process is recommended in order to minimize RNA carry-over into the DNA.

Step 3. DNA Precipitation

1. Precipitate DNA from the homogenate by the addition of 0.5 ml of 99.5% ethanol per 1 ml of ISOGENOME used for the isolation.
2. Mix samples and homogenate by inverting tubes 5-8 times.
3. Store at room temperature for 1-3 min.
4. DNA should quickly become visible as a cloudy precipitate. Transfer the DNA to a clean tube by spooling with a pipette tip. Swirl the DNA onto the tip and attach it to the tube wall near the top of the tube by gently sliding the DNA off the tip. *3

*3 If no precipitate is visible, or if it is too dispersed to easily collect with a pipette tip, centrifuge for 5 min at 5,000 x g and recover the pellet.

Step 4. DNA Wash

1. Add 1 ml of 75% ethanol and gently mix by inverting the centrifuge tube 3-6 times.
2. Store the tubes vertically for 0.5-1 min, or centrifuge for 1-2 min at 1,000 x g, to allow the DNA to settle to the bottom of the tubes and remove ethanol by pipetting or decanting.
3. Wash the DNA precipitate again (repeat 1-2 of Step 4). (During washes, DNA can be stored in 95% ethanol for at least one week at room temperature or for 3 months at 4°C.)

Step 5. DNA Solubilization

1. Remove any remaining ethanol from the bottom of a tube using a pipette.
2. Dissolve DNA in 8 mM NaOH or ddH_2O. [*4]
   The DNA preparations isolated from tissues such as liver, muscles contain some insoluble material (mostly polysaccharides). Remove the insoluble material by centrifugation at 12,000 x g for 10 min.
3. DNA samples solubilized in 8 mM NaOH is neutralized with HEPES. [*5]

*4 Add an adequate amount of 8 mM NaOH or ddH_2O to approach a DNA concentration of 0.2-0.3 µg/µl. Typically, add 0.2-0.3 ml of 8 mM NaOH or ddH_2O to the DNA isolated from 10^7 cells or 10-20 mg animal tissue.

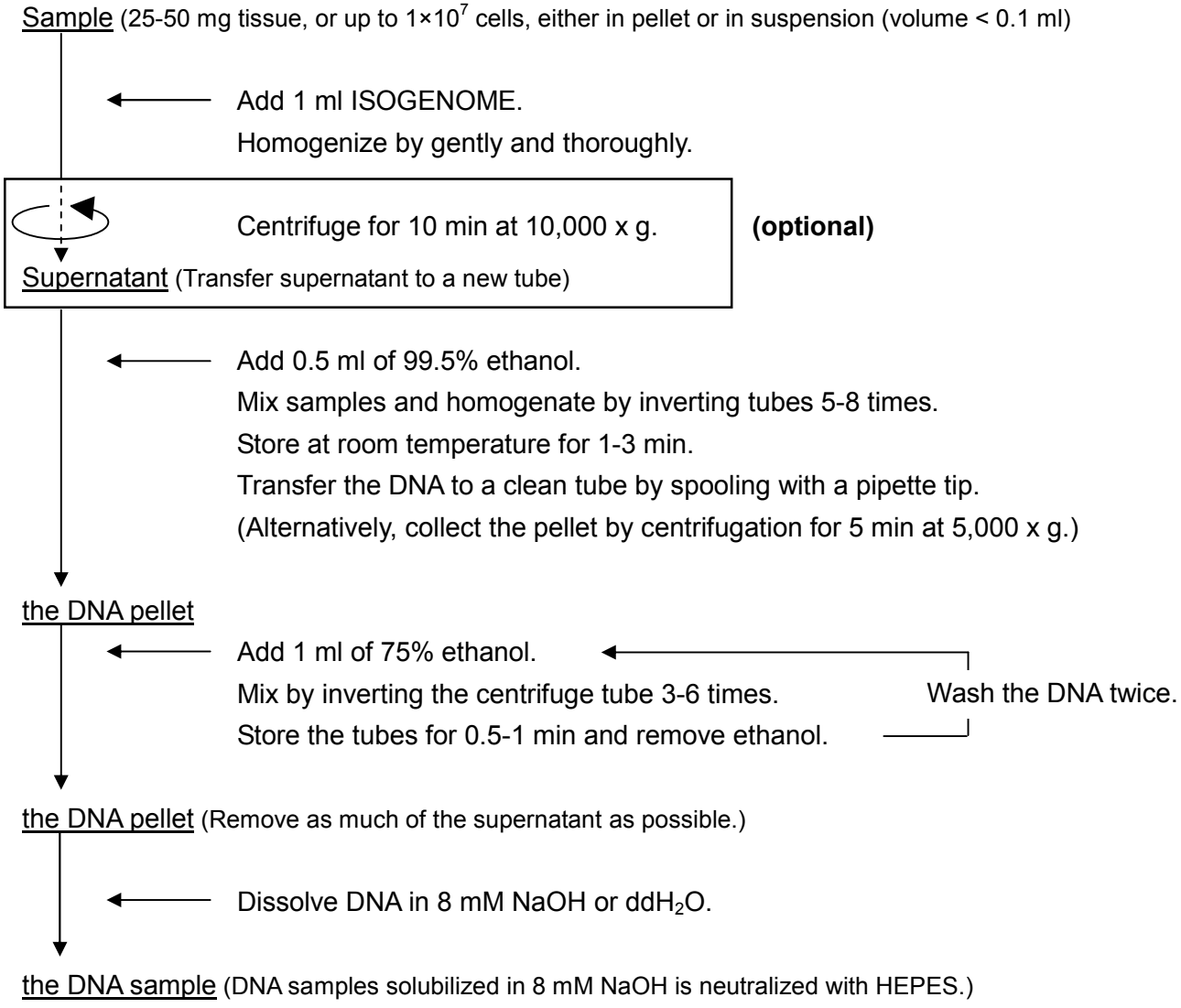
*5 For 1 ml of 8 mM NaOH use the following amounts of 0.1 or 1M HEPES (free acid).

＜Adjustment of pH in DNA samples solubilized in 8mM NaOH＞

Final pH	0.1 M HEPES
8.4	86 µl
8.2	93 µl
8.0	101 µl
7.8	117 µl
7.5	159 µl

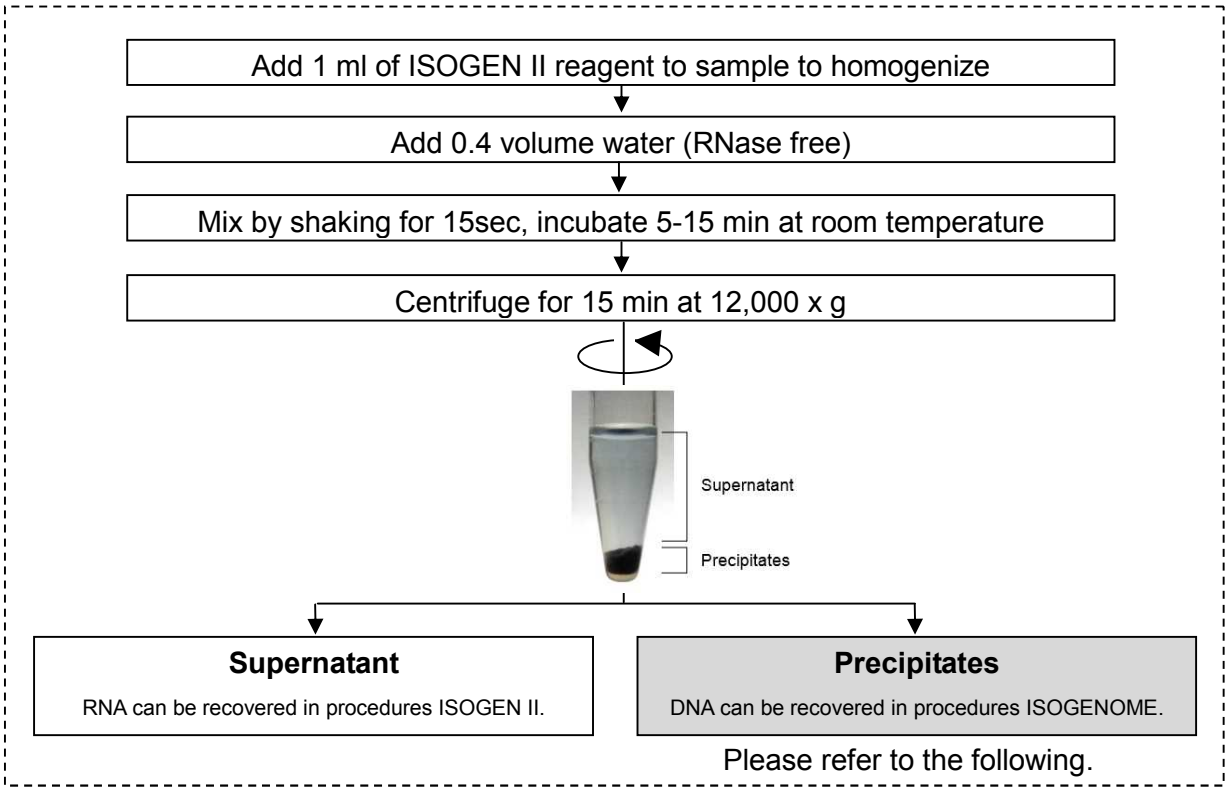
Final pH	1 M HEPES
7.2	23 µl
7.0	32 µl

＜Flowchart for Extraction of Genomic DNA＞



＜Protocol B: DNA and RNA extraction combined with ISOGEN II＞

ISOGENOME can be isolated of DNA (for use in PCR) and RNA from same sample by the use of combination with RNA extraction regent ISOGEN II (Code No. 311-07361).



Step 1. Lysis of Sample (Homogenization)

1. Remove any residual supernatant without disturbing the precipitate obtained after centrifugation of the ISOGEN II containing homogenate.
2. Vigorously mix the pellet with 8 - 10 volumes of ISOGENOME and incubate for 5-10 min at room temperature.

Perform steps 2 and later of Protocol A.

VI Rerated products

Code No.	Product Name	Size
318-08111	ISOGENOME	10 ml
314-08113		100 ml
317-07363	ISOGEN II	10 ml
311-07361		100 ml
315-02504	ISOGEN	10 ml
317-02503		50 ml
311-02501		100 ml
317-02623	ISOGEN-LS	10 ml
311-02621		100 ml
318-01793	Ethachinmate	0.02 ml
312-01791		0.2 ml
316-90101	Distilled Water, Deionized, Sterile	100 ml
318-90105		500 ml
312-90103		100 ml × 6
314-90021	TE（pH 8.0）	100 ml
316-90025		500 ml
310-90023		100 ml × 6

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If you have any questions, please contact us by web form.
The information in the descriptions of the products may be changed without prior notification.