Nippon Gene

ISOTISSUE

Code No. 317-03103 (For 3 Extractions), 311-03101 (For 30 Extractions)

Manual (ver.4) 20120106KH

I Product description

ISOTISSUE is a kit for extracting the genomic DNA from animal tissues. Since hazardous materials such as phenol and chloroform are not used during the handling and also a micro centrifuge tube is used, the handling is simple. The entire process can be completed in about 3 hrs. The genomic DNA obtained with this product is several 10 to several 100 kbp long and is highly purified so that it can be used as a substrate for restriction enzymes and for PCR.

II Precautions

• This product is a reagent for research and cannot be used for pharmaceutical or other purposes.
• 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 (www.nippongene.com).

III Contents of kit

Storage temperature: −20°C

	(3 extractions)	(30 extractons)
1. Extraction Buffer (*1)	1 ml × 2	20 ml
2. 20 mg/ml Proteinase K	150 µl	1.5 ml
3. 5 M NaCl	0.7 ml	7 ml
4. TE (pH 8.0)	1 ml × 2	20 ml
5. 10 mg/ml RNase A (*2)	20 µl	0.2 ml
6. 3 M Sodium Acetate (pH 5.2)	0.2 ml	1 ml × 2
7. 3 M LiCl (*3)	0.7 ml	7 ml
8. 150 mM Sodium Citrate (*3)	0.7 ml	7 ml

This product does not include isopropanol, ethanol or sterilized water.

(*1) When SDS is deposited in the Extraction Buffer, melt the SDS by incubating at 37°C before use.

(*2) White precipitates may be deposited in the RNase A solution but this does not indicate a quality or performance problem. In such cases, centrifuge and use only the supernatant.

(*3) Prepare the Wash Solution just before use.

Wash Solution composition (final concentration):
0.3 M LiCl, 15 mM Sodium Citrate, 75% Ethanol

Preparation method (For 30 extractions):
Prepare by adding 3.5 ml of sterilized water and 52.5 ml of Ethanol (99.5%) to each of 7 ml solutions of 3 M LiCl and 150 mM Sodium Citrate included in the kit. The prepared solution will become cloudy. After preparing, store the solution at −20°C.

IV Examples of use

Sample (Tissue) 50 mg (1.5 ml microtube)

← Extraction Buffer 500 µl
Homogenize (*1)
← 20 mg/ml Proteinase K 40 µl
Vortex thoroughly
Incubate at 55°C for 15 min, while stirring once or twice (*2)
← 5 M NaCl 200 µl
Vortex for 1-2 sec.
(so that added NaCl becomes as homogeneous as possible)
Incubate at 55°C for 5 min (*3)
Leave standing on ice for 5 min (Precipitates containing SDS are formed)
Centrifuge (12,000×g, for 10 min, 4°C)

↓

Supernatant (*4)

← Equal volume of isopropanol
Mix at room temperature for 5 min by gently inverting (*5)
Centrifuge (2,000×g, for 1 min)

↓

Precipitates

← TE (pH 8.0) 400 µl
Dissolve by tapping (*6)
← Add 5 µl of 10 mg/ml RNase A, vortex for 1-2 sec.
Incubate at 55°C for 15 min.
← Add 5 µl of 20 mg/ml Proteinase K, vortex for 1-2 sec.
Incubate at 55°C for 15 min.
← Add 40 µl of 3 M Sodium Acetate (pH 5.2), vortex for 1-2 sec.
← Add 440 µl of isopropanol (*7), vortex for 1-2 sec.
Centrifuge (2,000×g, for 1 min)

↓

Precipitates

← Add 1 ml of the Wash Solution (*8) and wash (*9)
← Add 1 ml of the Wash Solution, and wash once more (*9) (*10)
← Wash (*9) with 1 ml of 70% ethanol.
← Wash (*9) with 1 ml of ethanol (*11).
Air dry (*12) for 5-10 min.
← Dissolve (*13) with 200-500 µl of TE (pH 8.0).

↓

genomic DNAsolution (*14)

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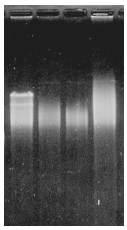
*1) The tissue may be minced beforehand with sterilized scissors or the like or homogenized with a Polytron Homogenizer.
*2) If the tissue is not completely dissolved, extend the incubation time with Proteinase K by 15-20 min. However, avoid extending Proteinase K incubation time by 45 min or longer.
*3) When the solution cannot be homogeneous due to high viscosity, extend the incubation time for another 5 min.
*4) When the supernatant and the precipitates cannot be completely separated by centrifugation, withdraw only the supernatant so that the jelly-like lumps and tissue residues are not included. However, if separating the supernatant and the precipitates is impossible, it may be advisable to interrupt the extraction and switch to a method in which extraction is performed after homogenizing thoroughly in step *1).
*5) Mix thoroughly and as gently as possible so that the chromosomal DNA is not damaged.
*6) When the precipitates cannot be dissolved completely, incubate at 55°C for 15 min and gently tap the tube with a fingertip to dissolve. Further, if turbidity remains after dissolving, centrifuge (12,000 × g, 15 min), withdraw the supernatant so as not to collect the precipitates and use as a sample.
*7) In place of isopropanol, 2.5 volumes of ethanol can be used.
*8) See "III Kit content" (*3) for the preparation method of the Wash Solution.
*9) After mixing by inverting, carefully remove the supernatant using a Pipetman so as not to suck in DNA precipitates.
*10) If contamination by polysaccharides and the like is suspected, the frequency of washing by the Wash Solution may be increased further.
*11) To make drying easy, this step may be omitted.
*12) Do not dry using a vacuum drier. If the precipitates are completely dried, the solubility is drastically reduced.
*13) Almost completely solubilized by standing overnight at around 4°C. If not dissolved after standing at 4°C, incubate at 55°C. If not solubilized by this treatment, gently pipette using a pipette tip with a larger bore by cutting the tip end at 2-3 mm (high molecular weight (>100 kb) DNA is fragmented to 20 and several kb by vigorous pipetting).

*14) Yield of genomic DNA obtained from mouse tissue

Mouse Tissue	DNA（μg/mg tissue）
Liver	0.5 ～ 1
Kidney	0.2 ～ 1
Heart	0.5 ～ 1.5

V Data

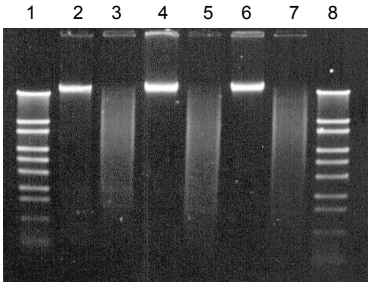
1） Genomic DNA obtained from mouse tissue



1. T4GT7 DNA : 166 kbp
2. Liver genomic DNA
3. Kidney genomic DNA
4. Heart genomic DNA

Pulse field gel electrophoresis

2) Restriction enzyme treatment of genomic DNA obtained from mouse tissue



1. Marker 6 (λDNA / *Sty* I)
2. Heart genomic DNA intact
3. Heart genomic DNA / *Eco*R I
4. Stomach genomic DNA intact
5. Stomach genomic DNA / *Eco*R I
6. Kidney genomic DNA intact
7. Kidney genomic DNA / *Eco*R I
8. Marker 6 (λDNA / *Sty* I)

Agarose S 0.8% gel, TAE Buffer 50V 80 min.

VI Troubleshooting

Q1. The sample is not dissolved or is difficult to dissolve.
A1. ・ Before adding the Extraction Buffer, mince the sample as finely as possible using sterilized scissors or the like.
・ Homogenize the sample with a Polytron homogenizer or the like.
・ Increase the volume of Proteinase K to be added.
・ Use the sample after diluting by adding twice the normal volume of the Extraction Buffer. However, depending on the sample DNA may be degraded.
・ Extend the incubation time of the Proteinase K treatment.
・ The sample may be heat denatured. Lower the temperature of the Proteinase K treatment to an appropriate temperature and extend the reaction time (For example, 37°C for 3 hrs).

Q2. Yield is low.
A2. ・ If the sample is not completely dissolved, dissolve according to A1 above.
・ If jelly-like material is found in the centrifuge fraction after the Proteinase K treatment, increase the volume ratio of the Extraction Buffer to the sample two-fold, homogenize and then extract.

Q3. OD_{260} / OD_{280} value is not more than 1.7.
A3. ・ Repeat washing DNA precipitates with the Wash Solution (0.3 M LiCl, 15 mM Sodium Citrate, 75% Ethanol).
・ If no effect is observed, add to the DNA solution 300 mM LiCl at final concentration and 150 mM Sodium Citrate at final concentration. Incubate at 55°C for 15 min and then perform the ethanol precipitation and wash with the Wash Solution (0.3 M LiCl, 15 mM Sodium Citrate, 75% Ethanol).

Q4. DNA is not dissolved.
A4. ・ Reduce the frequency of homogenization.
・ Increase the volume of the Extraction Buffer to be added.
・ Perform gentle pipetting (Use a pipette tip with a larger bore by cutting the tip end at 2-3 mm).

Q5. DNA is broken down.
A5. ・ Avoid pipetting as much as possible.
・ Perform pipettng as gently as possible (Use a pipette tip with a larger bore by cutting the tip end at 2-3 mm).
・ Use fresh samples.

Q6. RNA is contaminated.
A6. ・ Redo, starting with the RNase treatment.