Kit for RNA Extraction from Paraffin-Embedded Tissue Sections

ISOGEN PB Kit

Manual (Second edition)

Code No. 315-06421

NIPPON GENE CO., LTD.

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I Product description

The ISOGEN PB Kit is for extracting RNA from paraffin-embedded tissue sections.
RNA can be extracted in a short time (about 2 hours) by the following simple manipulations:
Deparaffinization → Proteinase K treatment → RNA extraction (ISOGEN-LS).

■ Use of extracted RNA

- Detection of RNA virus by RT-PCR.
- Detection of mRNA by RT-PCR.
- Checking the preservation of RNA in paraffin-embedded tissue sections, etc.

II Before using

Since RNA extracted from paraffin-embedded tissue sections is progressively degraded in the process before the embedding, the quality of the RNA is less than that extracted from fresh tissue.

The total RNA extracted from the fresh tissue of a mammal demonstrates clear bands of 28S rRNA and 18S rRNA in electrophoresis, and the ratio is about 2: 1. However, when the RNA is extracted from paraffin-embedded tissue, the result is not necessarily the same. Actually, in an extraction experiment using paraffin-embedded tissue of a mouse, almost no 28S rRNA was found. Therefore, it is suggested that the RNA is degraded in the process before the embedding. Further, the extent of RNA degradation is greatly dependent on the condition of the paraffin-embedded block. The presence of 18S rRNA is clearly confirmed in some blocks but in other blocks the degradation is progressed so much that the presence of 18S rRNA cannot be confirmed.

Therefore, we consider the persistence of 18S rRNA as one of the criteria of judgment. We recommend this as a guide, if the preservation of RNA is important in cases such as *In situ* hybridization.

Further, even if RNA is degraded (even if 18S rRNA is not confirmed), often the gene can be detected by RT-PCR, and normally the completeness of RNA is not required. However, since the amplification efficiency of the PCR from paraffin-embedded tissue sections is lower compared to normal PCR, make sure to set the primers so that the amplified size is not too big (not more than 400 bp, and preferably not more than 200 bp).

III Contents of kit

(For 20 extractions)

Proteinase K (20 mg/ml)	100 µl	× 1
Extraction Buffer *	3 ml	× 1
ISOGEN-LS	10 ml	× 1
Ethachinmate	60 µl	× 1
Deoxyribonuclease (RT Grade)	20 µl	× 1
10 × DNase (RT Grade) Buffer Ⅱ	100 µl	× 1
Stop Solution (RT Grade)	100 µl	× 1
DEPC treated Water	500 µl	× 2

* White crystals may appear in Extraction Buffer but this is not a quality problem.
In such cases, incubate at 37-50°C to completely dissolve the crystals and then use.

IV Storage

Store at －20°C

- Extraction Buffer can be stored at room temperature.
- ISOGEN-LS can be stored at 2-10°C.

V Precautions

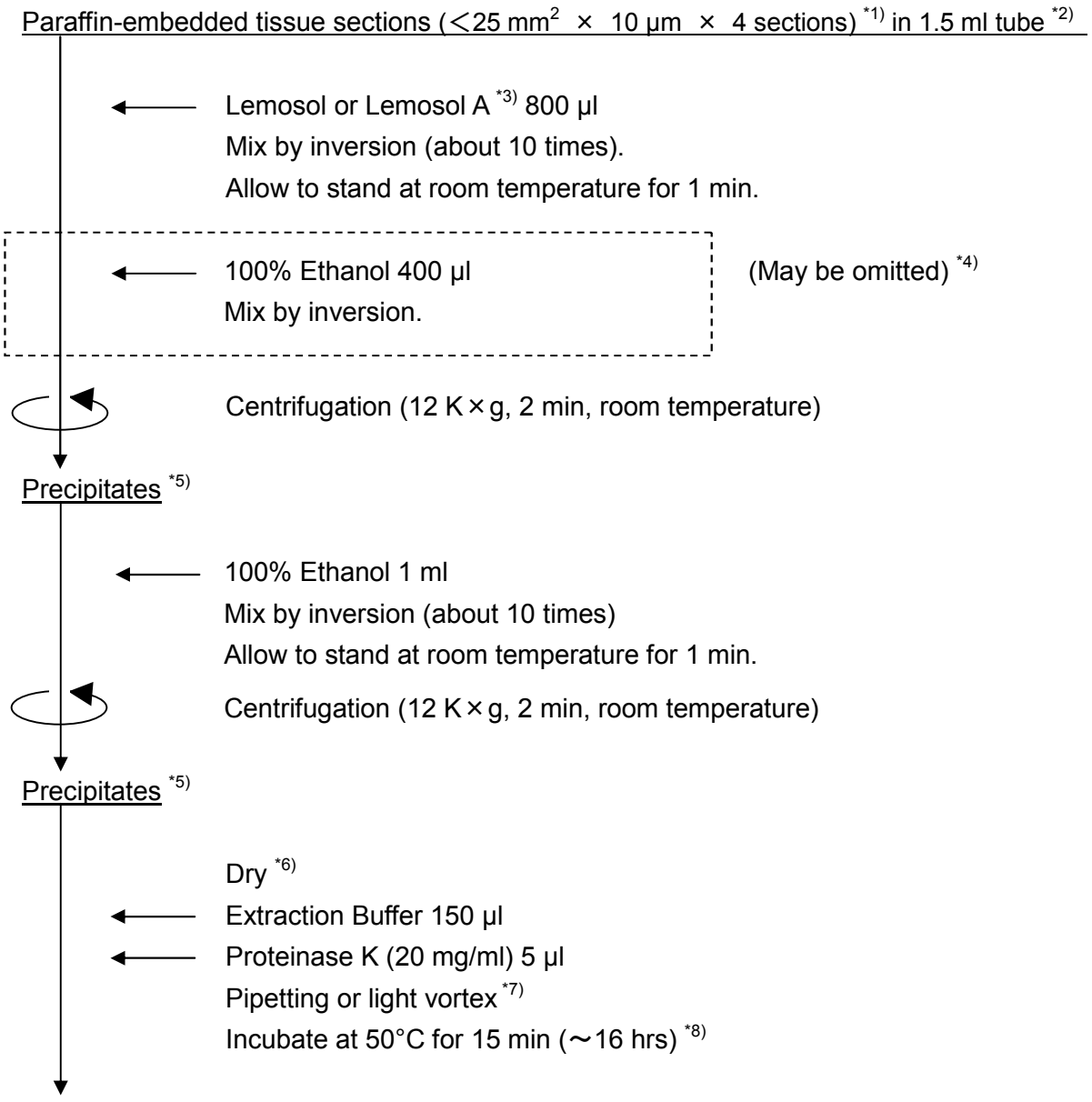
- This product is a reagent for research and cannot be used for medical or other objectives. Also, this product should be handled only by persons having a basic knowledge about reagents.
- ISOGEN-LS is a deleterious substance not for medical use (phenol formulation), and should be handled with great care.
- When using, wear appropriate protective gear (gloves, goggles, etc.).
- Ventilate the area well so as not to inhale the vapor.
- If the product enters eyes 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 https://www.nippongene.com/

VI Protocol

< Reagents necessary in addition to the kit >

- ☐ Lemosol or Lemosol A (Xylene may be used)
- ☐ Ethanol
- ☐ Chloroform
- ☐ Isopropanol

< RNA extraction protocol >



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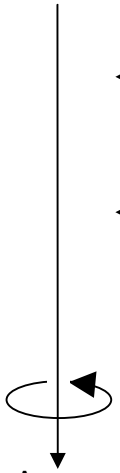
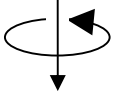


← ISOGEN-LS 450 µl
vortex 5 sec (～20 sec) [*9)]
Allow to stand at room temperature for 5 min.
← Chloroform 120 µl
vortex 15 sec
Allow to stand at room temperature for 3 min.

Centrifugation (12 K × g, 15 min, 4°C)

Aqueous phase (350 µl)

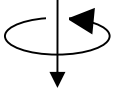
← Ethachinmate [*10)] 3 µl
← Isopropanol 300 µl
vortex 5 sec
Allow to stand at room temperature for 5 min (～10 min).



Centrifugation (12 K × g, 10 min, 4°C)

Precipitates

← 70% Ethanol 1 ml
Mix by light inversion



Centrifugation (12 K × g, 5 min, 4°C)

Precipitates

Dry [*11)]
← DEPC treated Water ～20 µl

RNA solution

*1) About 4 pieces of paraffin-embedded tissue sections (＜25 mm^2 × 10 μm) are used per extraction. Remove excess paraffin around the tissue using a utility knife or the like, then slice the tissue. Since paraffin is soluble in Lemosol, Lemosol A and Xylene, it is not necessary to completely remove paraffin.
Also, when RNA is extracted from a tissue section on a slide glass, scrape the tissue from the slide glass using a microspatula or the like before deparaffinization.

*2) We recommend use of 1.5 ml volume tubes made of clear polypropylene. Before use confirm that the tubes can withstand centrifugal force (12 K×g) and ISOGEN-LS (Phenol).

*3) Xylene may be used but we recommend use of Lemosol or Lemosol A because for improved safety.

*4) At this step, ethanol addition may be omitted. When the sample tends to float in the supernatant, the ethanol addition allows tissue to more easily precipitate and makes manipulation easier.
However, when a sample containing a large amount of paraffin is used, the ethanol addition may cause deposition of paraffin (white turbidity). Therefore, we recommend centrifuging without adding ethanol. If paraffin deposition is observed, centrifuge the sample to remove the supernatant, and then add Lonosol or Lemosol A again and perform the manipulation thereafter without adding “100% Ethanol 400 μl”.

*5) Remove the supernatant with a micropipette. Since the tissue may float at this step, be careful not to suck in.

*6) Air dry or vacuum dry until Ethanol is not visible.

*7) Make sure that the solution is foamy.

*8) RNA extraction efficiency may be improved by prolonging the reaction time of Proteinase K when a sample having a large tissue size is used, or when RNA yield is low.

*9) Mix until Extraction Buffer and ISOGEN-LS become homogeneous.

*10) Ethachinmate is an acrylamide polymer carrier solution used for precipitating nucleic acid with ethanol. RNA can be efficiently recovered by adding Ethachinmate, and the precipitation can be visually confirmed.

*11) Air dry ethanol until Ethanol is no longer visible. However, when RNA precipitates are completely dried, RNA may become difficult to dissolve.

< Handling of extracted RNA >

- When electrophoresis and concentration measurement are performed, use the preparation as-is.
- When the RNA prepared by this product is used for RT-PCR, treat the sample with Deoxyribonuclease (RT Grade) attached with the kit.

< Preparation of template RNA solution for RT-PCR: DNase treatment >

Extracted RNA solution	(0.1 - 5 µg)
10 × DNase (RT Grade) Buffer Ⅱ	2.5 µl
Deoxyribonuclease (RT Grade)	0.5 µl
DEPC treated Water	
Total	25 µl [*1)]

↓

Enzyme reaction 37°C 15 min [*2)]

↓ [*3)]

↓

Stop Solution (RT Grade) 2.5 µl

↓

Heat treatment 70°C 10 min [*4)]

↓

RT-PCR [*5)]

*1) When the reaction is carried out in a system other than 25 µl, the ratio of Deoxyribonuclease (RT Grade): 10 × DNase (RT Grade) Buffer Ⅱ : Stop Solution (RT Grade) should always be the volume ratio of 1: 5: 5. The effect of Stop Solution (RT Grade) may not be sufficient in other ratios. It is very important to chelate Ca^{2+} in the reaction solution for stopping DNase reaction.

*2) Do not react for 15 min or longer.

*3) When stopping at this time, DNase should be inactivated and removed by a phenol/chloroform treatment. In such cases, manipulations after adding Stop Solution (RT Grade) are unnecessary (see next page).

*4) RNA is unstable at a high temperature. Perform the heat treatment at 70°C. At this temperature DNase is not completely inactivated but it is not a problem in the reverse transcription reaction.

*5) After the reverse transcription reaction, stop the reaction of the reverse transcription enzyme by a heat treatment at 95°C for 5 min.

< Precautions for DNase treatment >

- Remove DNA by the reaction described above as quickly as possible before RT-PCR.
- When RNA treated with DNase needs to be stored for a long period of time, perform the inactivation and removal of DNase by the phenol/chloroform treatment.
- When electrophoresis is performed for RNA treated with DNase, perform the phenol/chloroform treatment and recovery by ethanol precipitation of the RNA. The mobility is affected abnormally by the effect of 10×DNase (RT Grade) Buffer Ⅱ and Stop Solution (RT Grade).
- When DNase is inactivated and removed by the phenol/chloroform treatment, the addition of Stop Solution (RT Grade) and the heat treatment at 70°C for 10 min are unnecessary.

(Example of DNase inactivation by phenol/chloroform treatment)

Add 75 µl of DEPC treated water to 25 µl of DNase treated RNA solution to bring the volume to 100 µl, and then add an equal volume (100 µl) of phenol/chloroform [*1)] and mix.

↓

Centrifuge (12 K×g, 5-10 min, room temperature) and transfer the upper layer (aqueous phase) to a new tube.

↓

Add an equal volume (100 µl) of chloroform to the upper layer and mix.

↓

Centrifuge (12 K×g, 5-10 min, room temperature) and transfer the upper layer (aqueous phase) to a new tube.

↓

Add 1/10 volume (10 µl) of 3 M sodium acetate solution and 2.5 fold volume (250 µl) of ethanol to the upper layer, mix and allow to stand at room temperature for 5-10 min (or at -20°C for 30 min).

↓

Centrifuge (12 K×g, 10 min, 4°C) and remove the supernatant.

↓

Wash the precipitates with 70% ethanol (add about 1 ml, mix lightly by inversion, centrifuge and remove the supernatant).

↓

Air dry the precipitates for 5-10 min and dissolve in DEPC treated Water.

↓

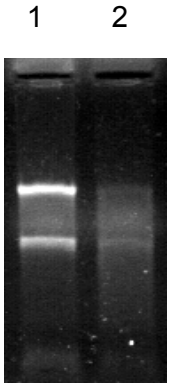
RNA solution

*1) "Phenol/Chloroform/Isoamyl alcohol (25: 24: 1)" (Code No. 311-90151)

VII Examples of experiments

< RNA extraction from paraffin-embedded mouse submaxillary gland sections >

RNA is extracted from 10 μm × 4 sections of paraffin-embedded mouse submaxillary gland according to the protocol and a quarter of the RNA solution (5 μl) is electrophoresed in 1% formalin denatured Agarose S.



1. RNA isolated from fresh tissue
   (extracted with ISOGEN)
2. RNA extracted from paraffin-embedded tissue sections
   (Extracted with ISOGEN PB Kit)

< Detection of the mouse glyceraldehyde-3-phosphate dehydrogenase (Gapd) gene by RT-PCR >

After treating 500 ng of the RNA extracted from paraffin-embedded mouse submaxillary gland sections with DNase according to the protocol, Exon 5 (258 bp) of the mouse Gapd gene was detected by RT-PCR.

Reverse transcription reaction

Reaction solution composition

10 × Reaction Buffer *	2 μl
25 mM $MgCl_2$ *	4 μl
2.5 mM dNTP *	4 μl
50 μM Random Nonamer *	1 μl
RNase Inhibitor (20 U/μl) *	0.5 μl
EuroScript RT (50 U/μl) *	0.5 μl
RNA treated with DNase	8 μl
Total	20 μl

Reverse transcription reaction condition

Total 20 μl

↓

25℃ 10 min

48℃ 30 min

95℃ 5 min

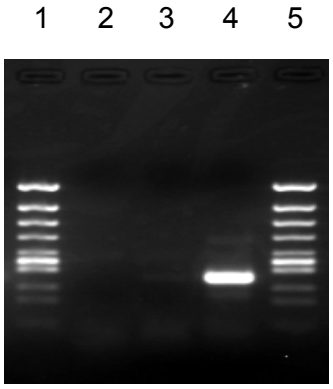
↓

1st strand cDNA

* Reverse Transcriptase qPCR Core Kit (EUROGENTEC INC.) was used for reagents of reverse transcription reaction.

PCR

Reaction solution composition		PCR condition	
10 × Gene *Taq* Universal Buffer	2.5 µl	94℃ 2 min	
2.5 mM dNTP Mixture	2 µl	95℃ 15 sec	× 35
3 µM Gapd Primer Mixture	2.5 µl	60℃ 30 sec	
Gene *Taq* NT (5 U/µl)	0.25 µl	72℃ 30 sec	
1st strand cDNA	5 µl		
Distilled Water	12.75 µl		
Total	25 µl		



1: OneSTEP Marker 5 (ΦX174 / *Hin*cIIdigest)

2: No template

3: RNA for which no reverse transcription reaction was performed.

4: RNA for which reverse transcription reaction was performed.

5: OneSTEP Marker 5 (ΦX174 / *Hin*cIIdigest)

One half volume (12.5 µl) of the PCR product was electrophoresed in 2% Agarose S.

< RT-PCR for microscale sample >

When the extracted RNA cannot be confirmed in electrophoresis or is not more than 10 ng, we recommend performing a reverse transcription reaction by adding RTmate (Code No. 315-05941). The detection limit sensitivity of RT-PCR is increased by adding RTmate. Detailed information about RTmate such as the sequence can be referred on our website (http://www.nippongene.com/).

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ISOGEN PB Kit (ver.2)
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