

DNA extraction method using sodium iodide without phenol and chloroform

## DNA Extractor<sup>®</sup> Kit Series

The sodium iodide method is introduced in USP 40 - NF 35 2nd "NUCLEIC ACID - BASED TECHNIQUES - APPROACHES FOR DETECTING TRACE NUCLEIC ACIDS (RESIDUAL DNA TESTING)".



A widely used method to extract DNA from tissues derived from living body is to precipitate and collect DNA using phenol and chloroform. However, this method requires the use of phenol and chloroform, hazardous substances. This DNA extraction method uses sodium iodide known as a chaotropic agent instead of these organic solvents. Sodium iodide has protein denaturation action and solubilization action. Sodium iodide releases chaotropic ions (I<sup>-</sup>) into the solution; the ions increase water solubility of hydrophobic molecules in the sample, and its action to weaken hydrophobic bond contributes particularly to denaturation and solubilization of membrane proteins such as nuclear membrane. In addition, no solid phase extraction using silica carrier inhibits DNA loss by adsorption; moreover, the entire operation is performed in a single tube, realizing a high yield rate.

### Features

- Organic solvents such as hazardous phenol and chloroform are not required.
- The entire process is performed in a single tube, causing no loss, contamination, or mechanical DNA damage in operation.
- No solid phase extraction using silica carrier causes no loss by adsorption.
- Little oxidation damage to DNA and mechanical DNA damage
- A high yield rate is realized.
- The product series used in the accumulated total of 963 publications \*<sup>1</sup>

\* 1: Source: Google scholar

### List of DNA Extractor<sup>®</sup> Kit series using the sodium iodide method

Sample	Product Name	Features
Viral DNA in serum/biological products	<b>DNA Extractor<sup>®</sup> Kit</b>	Low-cost, extraction of trace amount of DNA
Whole blood (human, cow, horse), cultured cells, tissues	<b>DNA Extractor<sup>®</sup> WB Kit</b>	Can extract high-molecular DNA
Genomic DNA in serum/plasma	<b>DNA Extractor<sup>®</sup> SP Kit</b>	Can remove blood-derived lipids
Oxidative stress marker 8-OHdG DNA	<b>DNA Extractor<sup>®</sup> TIS Kit</b>	Inhibits DNA oxidation in extraction process
Body hairs, nails, blood stains, saliva stain (forensic samples)	<b>DNA Extractor<sup>®</sup> FM Kit</b>	DNA extraction from trace amount of samples

**Extraction of residual DNA in biological products and viral DNA in serum**

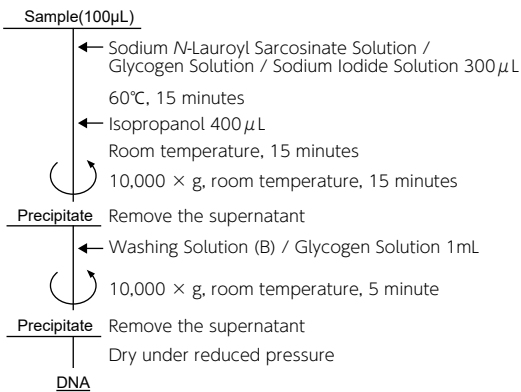
# DNA Extractor<sup>®</sup> Kit

Wako Cat. No.	Package Size
295-50201	50 tests

This kit is intended for use in extracting host cell-derived residual DNA remaining in biological components or viral DNA in serum using the Sodium Iodide method. The extracted DNA can be quantified using qPCR or the Threshold Immunoassay System<sup>®</sup> provided by Molecular Devices, LLC.

Using DNA Extractor<sup>®</sup> kit is recommended for testing and management of the amount of DNA derived from host cells such as CHO cells, *E. coli* and yeast.

## Extraction from serum



Operation time: 1 to 1.5 hours  
Sample amount: 100 µL/test

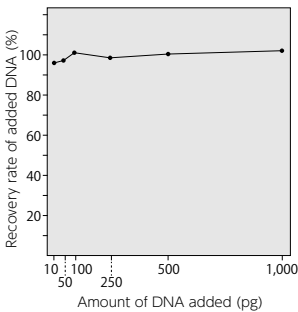
- Features**
- Infinitesimal amount of DNA (10 pg) can also be collected at a high yield of about 90%.
  - Organic solvents such as phenol and chloroform are not required.
  - The entire operation is completed in a single tube.
  - The operation is completed in a short time (1 to 1.5 hours).

- Kit Contents [50 tests (sample 100 µL)]:**
1. Sodium iodide solution.....26 mL × 1
  2. Sodium N-Lauroyl sarcosinate solution.....1.2 mL × 1
  3. Washing solution (A).....42 mL × 1
  4. Washing solution (B).....40 mL × 2
  5. Glycogen solution.....0.1 mL × 1

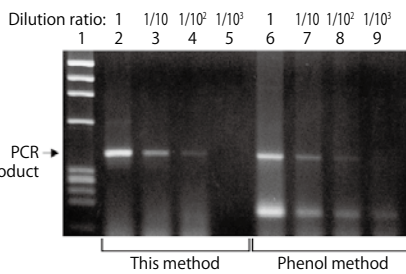
- Principle of DNA extraction**
- i. Proteins and lipids in samples are made soluble with sodium iodide and surfactant.
  - ii. Then, isopropanol is added to the samples to co-precipitate DNA with glycogen.
  - iii. At that time, the actions of sodium iodide, a chaotropic ion, and surfactant prevent the precipitation of components such as proteins in the samples, resulting in selective precipitation of DNA and glycogen.

## DNA Extractor<sup>®</sup> Kit Data

### Examples of using DNA Extractor<sup>®</sup> Kit



**DNA addition/recovery test**  
λ/Hind III 10 to 1,000 pg was labeled with <sup>32</sup>P and added to 100 µL of human serum. DNA was extracted using this kit and the recovery rate was calculated.



**Detection by PCR**  
Serum containing hepatitis B virus (HBV) was diluted in stages. DNA was extracted using this kit and the phenol method. HBV-DNA was amplified with PCR, and DNA was detected using agarose gel electrophoresis.  
Lane 1: Marker DNA (ϕ X174/Hae III)

**Reference** Ishizawa, M. *et al.*, *Nucleic Acids Res.*, **19**, 5792 (1991).

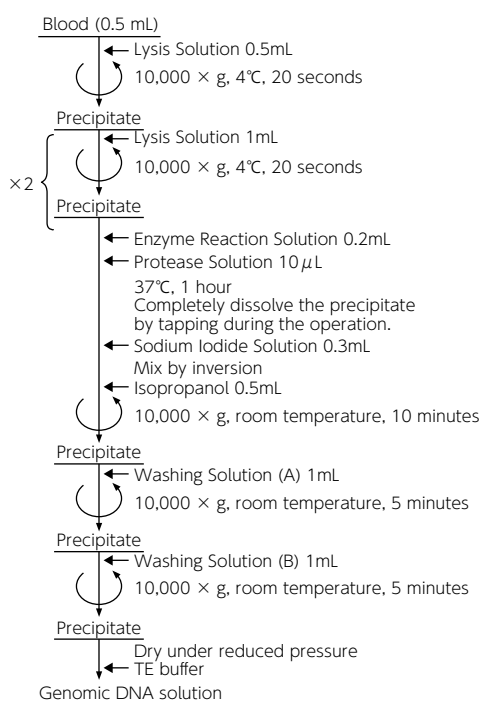
**Genomic DNA extraction from human whole blood sample**  
**Extraction from cow/horse blood, cultured cells, and tissues is also possible**

# DNA Extractor<sup>®</sup> WB Kit

Wako Cat. No.	Package Size
291-50502	50 tests

Human genomic gene analysis requires the preparation of high-molecular genomic DNA. Although many types of genomic DNA extraction methods are practiced at present, most of them use organic solvents such as phenol. Its toxicity and extraction procedures requiring time and effort are the disadvantages.

This kit is primarily for the extraction of genomic DNA from human whole blood. A high yield of DNA with high purity can be obtained in a single tube in a short time without using hazardous phenol and chloroform.



Operation time: 1.5 hours  
 Sample amount: 500 µL/test

- Features**
- A high yield rate of high-molecular DNA of over 90%
  - The entire process is completed in a single tube in about 1.5 hours.
  - Organic solvents such as phenol and chloroform are not required.
  - Extraction from human whole blood as well as cow/horse blood, cultured cells, and tissues is also possible.
  - The operation is easier than the phenol/chloroform extraction method and suitable for processing multiple samples.
  - The entire procedure is performed in a single tube, reducing genomic DNA damage by mechanical cutting in operation.

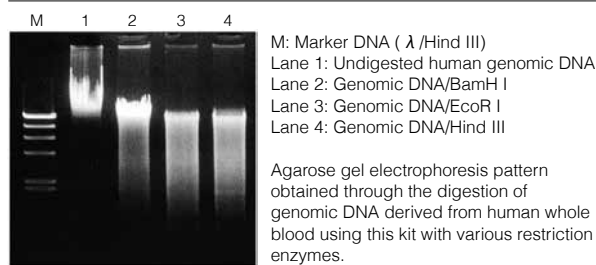
**Kit Contents [50 tests (sample 500 µL)]:**

1. Lysis solution	65 mL × 2
2. Enzyme reaction solution	10 mL × 1
3. Sodium iodide solution	15 mL × 1
4. Washing solution (A)	50 mL × 1
5. Washing solution (B)	50 mL × 1
6. Protease	10 mg × 1

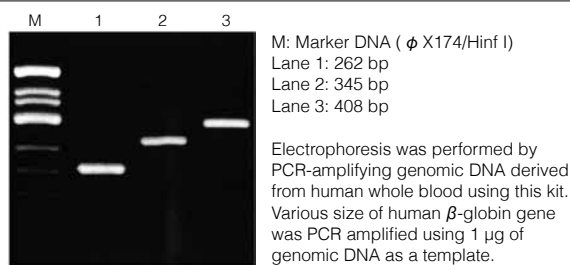
- Principle of DNA extraction**
- i. Cell membrane and cytoplasm are destroyed by surfactant, isolating cell nuclei.
  - ii. The nuclear membrane and nuclear protein are decomposed by protease to expose DNA.
  - iii. Protein and lipids are made soluble by the action of sodium iodide, and DNA is precipitated with isopropanol.

## DNA Extractor<sup>®</sup> WB Kit Data

### Examples of using DNA Extractor<sup>®</sup> WB Kit

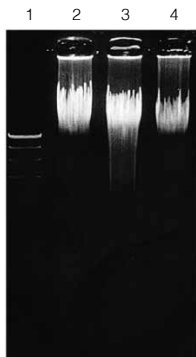


Reference Wang, L. *et al.*, *Nucleic Acids Res.*, **22**, 1774 (1994).



## Genomic DNA extraction from cow/horse whole blood

Genomic DNA with high purity can be efficiently extracted from cow/horse whole blood by partially changing the standard protocol. Protease treatment is performed for 2 hours for horse blood, whereas washing operation with lysis solution is repeated 4 times for cow blood, followed by treatment with protease for 4 hours. The operation time is about 3 hours for horse blood and 5 hours for cow blood. Blood samples used were added with EDTA·2Na or heparin as an anticoagulant agent.



### Electrophoretic image of genomic DNA extracted from cow/horse

Lane 1:  $\lambda$ /Hind III  
Lane 2: Horse blood (EDTA·2Na)  
Lane 3: Horse blood (Heparin)  
Lane 4: Cow blood (EDTA·2Na)

3 $\mu$ L of 100  $\mu$ L extracted DNA solution was loaded on a 1% agarose gel and DNA was separated by electrophoresis.

### Operation procedure

#### Extraction from horse blood

Treatment with protease is performed for 2 hours until there is no nuclear pellet. Follow the standard protocol except for this operation.

#### Extraction from cow blood

This operation, which isolate cell nuclei and wash it by adding lysis solution, is repeated 4 to 5 times, because it is difficult to remove foreign substances such as hemoglobin only with 2 operations as described in the protocol. The reference is until the red pellets become transparent. Since pellets after centrifugation are very prone to peeling, sufficient attention is required in decantation. Also, perform treatment with protease for about 4 hours.

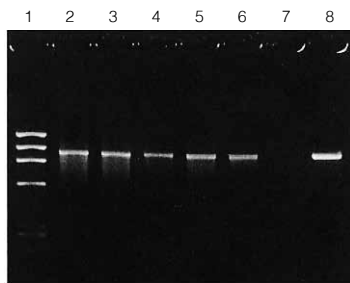
### Yield and purity of genomic DNA extracted from cow/horse

Sample	DNA yield ( $\mu$ g/mL blood)	$A_{260}/A_{280}$
Horse (EDTA·2Na)	56.62	1.91
Horse (Heparin)	48.34	1.88
Cow (EDTA·2Na)	22.04	1.86

Notes) Each horse blood sample was collected from different horses.

## DNA extraction from tissues

Genomic DNA can be extracted from tissues by adding 2 to 3 simple operations to the standard protocol. The operation is completed in about 2 hours. Resulting DNA has little contamination with RNA or protein and can be used in restriction enzyme treatment, PCR, Southern blotting analysis, fingerprinting, etc.



### Examples of rat gene detection by PCR

Rat GAPDH gene fragment (983 bp) was amplified using DNA extracted from each rat tissue as a template.

← 983bp  
Lane 1: Marker DNA ( $\phi$  X174/Hae III)  
Lane 2: Stomach  
Lane 3: Intestine  
Lane 4: Liver  
Lane 5: Pancreas  
Lane 6: Skeletal muscles  
Lane 7: Negative control  
Lane 8: Positive control

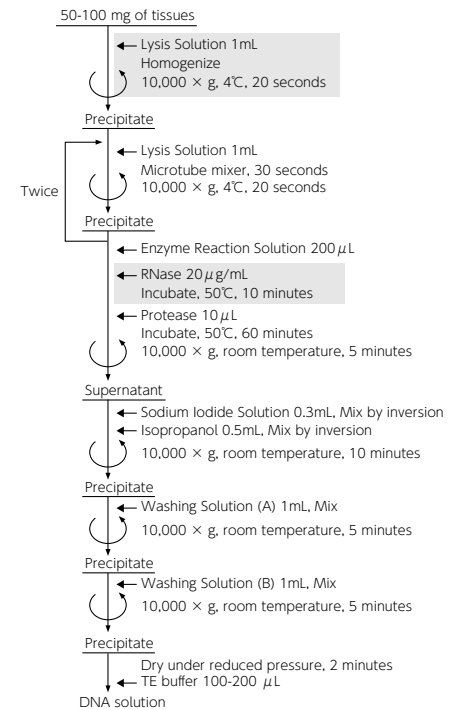
A band of 983 bp could be detected in all DNAs extracted from each tissue.

### Yield and purity of DNA extracted from each rat tissue using this kit

Tissues	DNA yield ( $\mu$ g/100 mg of tissues)	$A_{260}/A_{280}$
Stomach	175	1.92
Intestine	396	1.96
Liver	286	1.95
Pancreas	624	1.95
Skeletal muscles	67	1.85

Despite of variations in DNA yield per unit of tissues, DNA with high purity could be extracted.

### Protocol (DNA extraction from tissues)



# Q&A for DNA Extractor® WB Kit

## Q1. What is the principle of DNA Extractor® WB Kit?

This kit is for the extraction of genomic DNA from whole blood and cultured cells. Only cell nuclei are collected by adding a lysis solution containing surfactant first. Next, the nuclear membrane and foreign substances are decomposed by protease to release DNA. Then, after protein is made soluble with sodium iodide, isopropanol is added to precipitate and collect genomic DNA only. Also, with this method, the whole DNA extraction process can be performed in a single tube.

## Q2. Isn't it contaminated with RNA?

Although contamination with RNA is rare in extraction from whole blood, cultured cells will be contaminated with RNA more or less. In this case, RNA can be almost completely removed by newly adding the treatment with RNase at 37°C for 10 minutes to the basic protocol.

## Q3. The yield rate of DNA is low. Why?

The followings are the possible reasons.

### 1. First centrifugation operation

For the centrifugation operation at 10,000 × g for 20 seconds, perform centrifugation for 20 seconds after the centrifuge completely reached 10,000 × g. Cell nuclei will not completely detach in 20 seconds after the start button of the centrifuge is pressed; therefore, the DNA yield rate may drop by 20 to 30%. Although it varies by centrifuge, 10,000 × g is equivalent to approximately 12,000 rpm in case of a tabletop centrifuge.

### 2. Supernatant removing operation after centrifugation

Perform all operations by decantation. Removal with a pipette may result in a low yield of DNA.

### 3. DNA dissolution

As DNA in high concentration is extracted, add TE buffer (100-200 µL) and slowly dissolve it at room temperature for 3 hours or at 4°C overnight. DNA will not be completely dissolved in a short time, which may result in an apparent low yield.

## Q4. Can DNA be extracted from 100 µL of blood?

Yes. In this case, use the standard protocol by scaling it down. Also, when the amount of blood is 100 µL or less, add physiological saline to make up to 100 µL and perform the same procedure. One kit can be used 250 tests for 100 µL of blood.

## Q5. Can DNA be extracted by using heparin as an anticoagulant agent instead of EDTA?

DNA can be successfully extracted at a usual concentration of heparin (10 units/mL of blood) or less, but the yield rate markedly decreases from a concentration of around 20 units/mL. Also, DNA extracted from blood added with heparin may affect restriction enzyme treatment or PCR reaction. Therefore, the use of EDTA·2Na or EDTA·2K (1 mg/mL of blood) is recommended.

## Q6. Can DNA be extracted from stored blood?

DNA can be extracted successfully if blood is stored at 4°C for 3 weeks or less. However, in case of a long-term storage of 1 month or more, DNA will be gradually decomposed. Blood should be stored frozen in case of long-term storage.

## Q7. What should be done without a microtube mixer?

Mix the sample by inversion for about 1 to 2 minutes.

## Q8. We want to suspend the experiment.

Store the precipitate separated by centrifugation with the addition of lysis solution at -20°C.

## Q9. We do not have a refrigerated centrifuge.

Extraction can be performed with a tabletop centrifuge. In that case, perform the operation quickly.

## Q10. How much DNA can be extracted from 1 mL of blood?

Although there are variations by sample, approximately 20 to 70 µg of DNA can be extracted from normal humans.

## Q11. Can DNA be extracted from tissues with this kit?

Yes. DNA can be extracted by fragmenting tissues by homogenization.

## Q12. What is the initial cell number when extracting DNA from cultured cells?

10<sup>3</sup> to 10<sup>6</sup> cells will be enough for extraction. Cultured cells will be contaminated with RNA; therefore, before treatment with protease, add 20 µg/mL of RNase A and treat at 37°C for 10 minutes.

## Q13. Can mitochondrial DNA be extracted?

Yes. Please see the following publication.

Kubota, N., Hayashi, J., Inada, T. and Iwamura, Y.: *Radiation Research*, **148**, 395 (1997).

For the extraction of mitochondrial DNA alone, the use of mtDNA Extractor® WB Kit (Wako Cat. No. 293-54401) and mtDNA Extractor® CT Kit (Wako Cat. No. 291-55301) is recommended.

## Q14. What are the differences from DNA Extractor® Kit (Wako Cat. No. 295-50201)?

The purpose of use is different. DNA Extractor® Kit is for the extraction of a slight amount of viral DNA in serum and a slight amount of host cell-derived DNA in biological products, while DNA Extractor® WB Kit specifically extracts genomic DNA from whole blood and cultured cells.

## Q15. How should protease be stored after prepared?

It is stable at -20°C for 6 months.

## Q16. What are the differences from DNA Extractor® WB-Rapid Kit?

DNA Extractor® WB-Rapid Kit is an improved product to enable DNA extraction in about 20 minutes. It is suitable for treating many samples in a small volume.

## Q17. Some insoluble matters still remain after treatment with protease at 37°C for 1 hour. What should be done?

Failure to digest in an hour is considered attributable to insufficient washing with lysis solution in the first step. When cell nuclei are precipitated in the beginning, repeat washing until red pigment derived from hemoglobin is eliminated.

## Q18. When DNA extraction from cultured cells was performed, white insoluble matters emerged at the time of adding sodium iodide. Should we move on to the next step after removing these matters?

Insoluble matters may be observed depending on types of cultured cells. In this case, there is no problem to move to the next step, washing operation with washing solution (A), without removing the matters. Insoluble matters will be dissolved by adding washing solution (A). If the purity of resulting DNA is low, it is recommended to repeat the operation of cell nuclei precipitation by lysis solution.

## Q19. The A<sub>260</sub>/A<sub>280</sub> ratio of DNA is 1.8 or below. What should be done?

Sodium iodide may be remained in DNA. In this case, repeat washing with washing solution (B).

## Q20. Is there any reference literature?

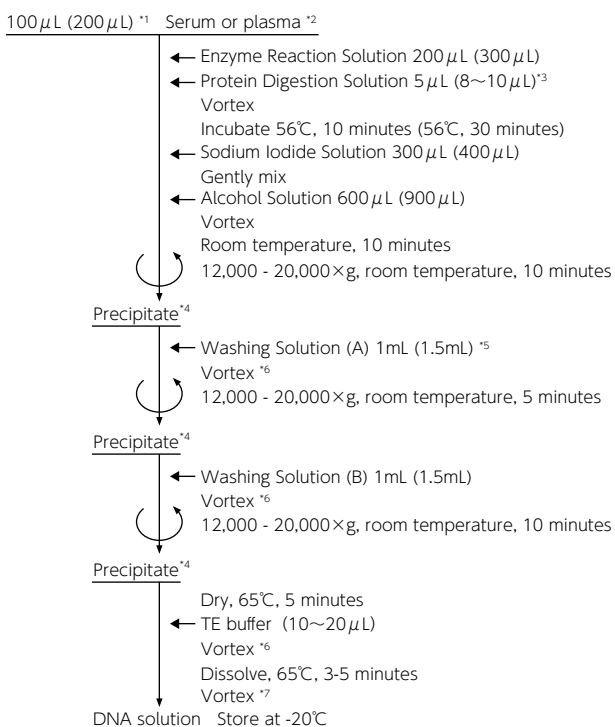
See the following reference: Wang, L., Hirayasu, K., Ishizawa, M. and Kobayashi, Y.: *Nucl. Acids Res.*, **22**, 1774 (1994).

# DNA Extractor<sup>®</sup> SP Kit

Wako Cat. No.	Package Size
296-60501	50 tests

This kit, an improved version of DNA Extractor<sup>®</sup> Kit, is a DNA extraction kit optimized for the extraction of DNA fragments in serum or plasma. It can obtain a very high DNA yield, so is useful as a pretreatment reagent to detect and analyze the target gene with a high sensitivity. Also, this product uses the sodium iodide method without using organic solvents such as phenol and chloroform.

## Methods



- \* 1 The protocol starting with 200  $\mu$ L of sample is shown in the parentheses.
- \* 2 Handle serum (plasma) samples on the ice. Add enzyme reaction solution as early as possible so that DNase in serum (plasma) will not be activated.
- \* 3 Take it out of the kit and transfer it on the ice before use.
- \* 4 Discard the supernatant, place the tube upside down on a paper towel, and remove remaining supernatant by pressing the mouth of the tube to the paper towel.
- \* 5 After washing solution (A) is added, the sample can be stored for a long term at -20°C.
- \* 6 Mix well until the precipitate peels off from the wall of the tube.
- \* 7 Dissolve completely until there is no precipitate.

- References**
- 1) Ishizawa, M. *et al.*, *Nucleic Acids Res.*, **22**, 1774 (1994).
  - 2) Sozzi, G. *et al.*, *Clin. Cancer Res.*, **5**, 2689 (1999).
  - 3) Silva, J. M. *et al.*, *Cancer Res.*, **59**, 3251 (1999).
  - 4) Shao, Z. M. *et al.*, *Clin. Cancer Res.*, **7**, 2222 (2001).

Operation time: 1 hour or more

Sample amount: 100  $\mu$ L/test

## Features

- DNA can be obtained at a high yield rate from a small amount (100  $\mu$ L) of serum and plasma sample.
- "Sodium iodide method" achieving nearly 100% yield rate is adopted.
- Organic solvents such as phenol and chloroform are not required.
- No use of solid phase extraction method using silica carrier realizes a high yield of DNA.
- High-quality DNA suitable for PCR can be obtained.
- Lipids, etc., derived from blood sample can be completely removed with specially prepared alcohol solution included in the kit.
- The entire operation is completed in a single tube.

## Kit Contents [50 tests (sample 100 $\mu$ L)]:

1. Enzyme reaction solution ..... 10 mL  $\times$  1
2. Protein digestion solution ..... 250  $\mu$ L  $\times$  1
3. Sodium iodide solution ..... 15 mL  $\times$  1
4. Alcohol solution ..... 30 mL  $\times$  1
5. Washing solution (A) ..... 50 mL  $\times$  1
6. Washing solution (B) ..... 50 mL  $\times$  1

## Principle of DNA extraction

- i. Protein derived from serum/plasma is decomposed by protease.
- ii. Protein and lipids derived from serum/plasma are made soluble by the action of sodium iodide and removed with alcohol solution included in the kit to precipitate DNA.





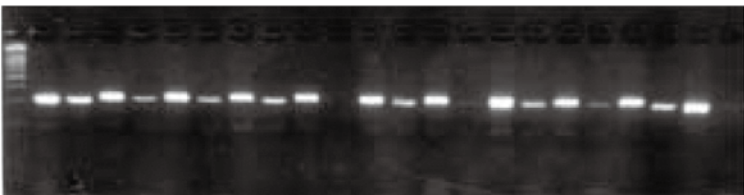
## DNA Extractor<sup>®</sup> SP Kit Data

### Examples of using DNA Extractor<sup>®</sup> SP Kit

#### Amplification of p53-Exon 5 region of DNA extracted from human serum and plasma

Using this kit, DNA was extracted from human serum (10 samples) and human plasma (1 sample) and dissolved in 20 µL of TE buffer (pH 8.0) to prepare DNA samples. The p53-Exon 5 region (308 bp) was amplified from 5 µL of each sample. The kit was compared with DNA extraction kit (Company A) which is based on the extraction principle with silica carrier (centrifugal filtration method) as control.

Serum 1 Serum 2 Serum 3 Serum 4 Plasma Serum 5 Serum 6 Serum 7 Serum 8 Serum 9 Serum 10  
M W A W A W A W A W A W A W A W A W A W A W A



M: DNA Step Ladder Mix (80-10 kbp)  
W: DNA Extractor<sup>®</sup> SP Kit  
A: Kit of Company A

← 308bp

PCR: 40 cycles  
3% Agarose

#### Method of extracting DNA from 1 to 5 mL sample using DNA Extractor<sup>®</sup> SP Kit

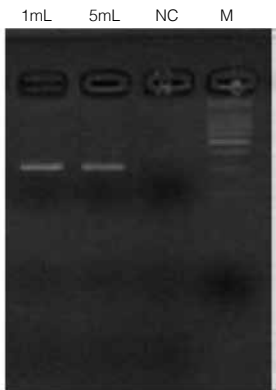
After dissolving in TE buffer, insoluble matters were precipitated and absorption was detected in the background (absorbance 320 nm), indicating lower purity than the standard method (extraction from 100- 200 µL). However, amplification by PCR can be performed successfully.

##### Method for treating 1 mL serum (plasma) sample

- 1 mL sample/15 mL centrifuge tube
- ← 2mL Enzyme Reaction Solution
- ← 50 µL Protein Digestion Solution
- 56°C, 30 minutes
- ← 3mL Sodium Iodide Solution
- ← 6mL Alcohol Solution
- Room temperature, 15 minutes
- 10,000 rpm, 20 minutes
- Precipitate
- ← 10mL Washing Solution (A)
- 10,000 rpm, 10 minutes
- Precipitate
- ← 10mL Washing Solution (B)
- 10,000 rpm, 10 minutes
- Precipitate
- (Dissolve in an appropriate amount of TE, etc.)

##### Method for treating 5 mL serum (plasma) sample

- 5 mL sample/50 mL centrifuge tube
- ← 7.5mL Enzyme Reaction Solution
- ← 250 µL Protein Digestion Solution
- 56°C, 30 minutes
- ← 10mL Sodium Iodide Solution
- ← 22.5mL Alcohol Solution
- Room temperature, 15 minutes
- 10,000 rpm, 20 minutes
- Precipitate
- ← 38mL Washing Solution (A)
- 10,000 rpm, 10 minutes
- Precipitate
- ← 38mL Washing Solution (B)
- 10,000 rpm, 10 minutes
- Precipitate
- (Dissolve in an appropriate amount of TE, etc.)



308bp →

NC: Negative Control  
M: Marker

The p53-Exon 5 region (308 bp) samples for PCR (35 cycles). Of DNA extracted from 1 mL and 5 mL of serum, DNA equivalent to 10 µL of serum was applied to PCR. The target DNA sequence was successfully amplified.



**DNA extraction for 8-OHdG measurement**  
**DNA extraction from human and animal parenchyma tissues**

# DNA Extractor<sup>®</sup> TIS Kit

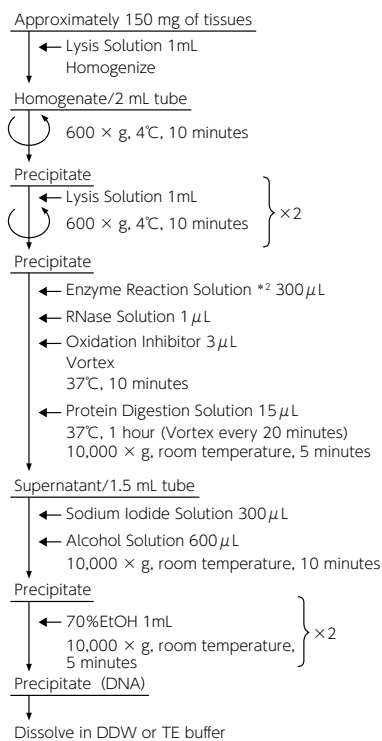
Wako Cat. No.	Package Size
296-67701	50 tests

The sodium iodide method, the basic principle of DNA Extractor<sup>®</sup> Kit series, is known as a DNA extraction method causing relatively little oxidation during operations. This kit further inhibits oxidation of DNA with the use of an oxidation inhibitor and is useful for the measurement of 8-OHdG (8-hydroxy-2'-deoxyguanosine), an oxidative stress marker.

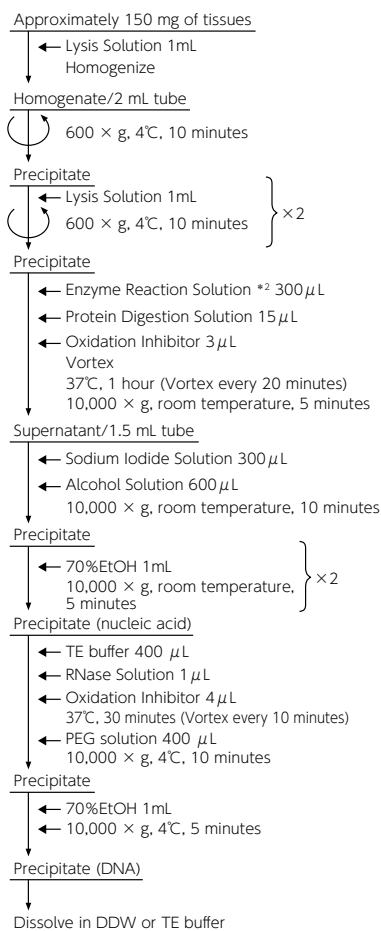
Operation time: 2 hours or more  
 Sample amount: 100 µL/test

## Methods

### Protocol 1



### Protocol 2\*1



**Protocol 1** is a simplified method where treatment with RNase is performed before treatment with protease.

**Protocol 2** is a method to collect DNA by polyethylene glycol (PEG) precipitation method where protease treatment and RNase treatment are performed in different processes to enhance the purity of DNA.

There is no difference in the amount of 8-OHdG between the two methods, and the levels of DNA oxidation inhibition are comparable.

\* 1 DNA purity can be enhanced with this protocol.

\* 2 If there is any deposit in enzyme reaction solution, completely dissolve the deposit by heating it to 37°C or returning it to room temperature and mix before use.

### Features

- Useful for the detection and measurement of oxidative stress markers. **Sodium iodide method causes little oxidation of DNA during operation. The use of oxidation inhibitor further inhibits oxidation of DNA.**
- Organic solvents such as phenol and chloroform are not required.
- Suitable for DNA extraction from human and animal parenchyma tissues.

### Kit Contents [50 tests (sample 100µL)] :

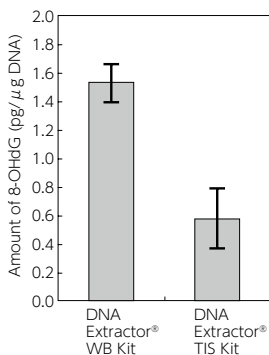
1. Lysis solution ..... 75 mL × 2
2. Enzyme reaction solution ..... 15 mL × 1
3. RNase solution ..... 50 µL × 1
4. Protein digestion solution ..... 750 µL × 1
5. Oxidation inhibitor ..... 350 µL × 1
6. Sodium iodide solution ..... 15 mL × 1
7. Alcohol solution ..... 30 mL × 1
8. PEG solution ..... 20 mL × 1



## DNA Extractor® TIS Kit Data

### DNA extraction from mouse liver and 8-OHdG measurement

DNA was extracted from mouse liver (the same mouse) using DNA Extractor® WB Kit [Wako Cat. No. 291-50502] and DNA Extractor® TIS Kit [Protocol 1]. Resulting DNA was treated with nuclease P1, the amount of 8-OHdG was measured by High Sensitive 8-OHdG Check [Wako Cat. No. 307-07921] and HPLC/ECD, and two methods were compared.



#### Measurement by High Sensitive 8-OHdG Check

The amount of 8-OHdG generated during operation could be reduced to half as compared with DNA Extractor® WB Kit. As a result, the kit showed superior performance as a DNA extraction kit for 8-OHdG measurement.

#### Measurement by HPLC/ECD

	8-OHdG concentration (nmol/L)	dG concentration (μmol/L)	8-OHdG/10 <sup>5</sup> dG
DNA Extractor® WB Kit	1.41	687	0.21
DNA Extractor® TIS Kit	0.59	692	0.08

#### [HPLC conditions]

Column: Wakosil-II 5C18 RS φ 4.6 mm × 150 mm  
 Eluent: 50 mmol/L sodium acetate (pH 5.2), 9% MeOH  
 Flow rate: 1 mL/min  
 Absorbance: UV 260 nm  
 ECD electrolytic potential: +600 mV

The amount of 8-OHdG generated during operation can be reduced to half or less as compared with DNA Extractor® WB Kit. Also, the level of 8-OHdG in deoxyguanosine has been revealed to be very low, showing superior performance as a DNA extraction kit for 8-OHdG measurement.

# 8-OHdG Assay Preparation Reagent Set

Wako Cat. No.	Package Size
292-67801	50 tests

When measuring 8-OHdG in tissues, extracted DNA from tissues need to be hydrolyzed. This product is a reagent kit including enzyme, buffer, etc., used in pretreatment of 8-OHdG measurement.

Add a specified amount of each reagent to DNA samples for measurement to promote reaction, thereby reducing variability in the nuclease treatment status (mononucleotide release) and realizing stable measurement of 8-OHdG.

#### Kit Contents [50 tests]:

1. Acetic acid buffer ..... 950 μL × 1
2. Nuclease P1 ..... 500 units × 1
3. Tris buffer ..... 1 mL × 1
4. Alkaline phosphatase solution ..... 50 μL × 1

#### Related product

This is an ELISA kit used to determine 8-OHdG amount, a biomarker of DNA oxidative damage.

NIKKEN SEIL Co, Ltd.

Wako Cat. No.	Manufacturer Cat No.	Product name	Package Size
307-07921	KOG-HS10/E	High Sensitive 8-OHdG Check	96 tests
301-06101	KOG-200S/E	New 8-OHdG Check	1 set

#### Methods

- (pretreatment before 8-OHdG measurement)
- DNA sample 200 μg/150 μL DDW
- ↓
- Treatment by boiling 98°C, 2 minutes
- ↓
- Rapid cooling (on ice), 5-10 minutes
- ← Acetic acid buffer 19 μL
  - ← Nuclease P1 solution 10 μL
- 37°C, 30 minutes
- ← Tris buffer 20 μL
  - ← Alkaline phosphatase solution 1 μL
- 37°C, 30 minutes
- ↓
- 10,000 MW ultrafiltration (spin filtration 15,000 × g, 20 minutes)
- ↓
- Filtrate (DNA concentration: 1 mg/mL)
- ↓
- 8-OHdG measurement (HPLC method or ELISA method)

## DNA extraction from forensic samples

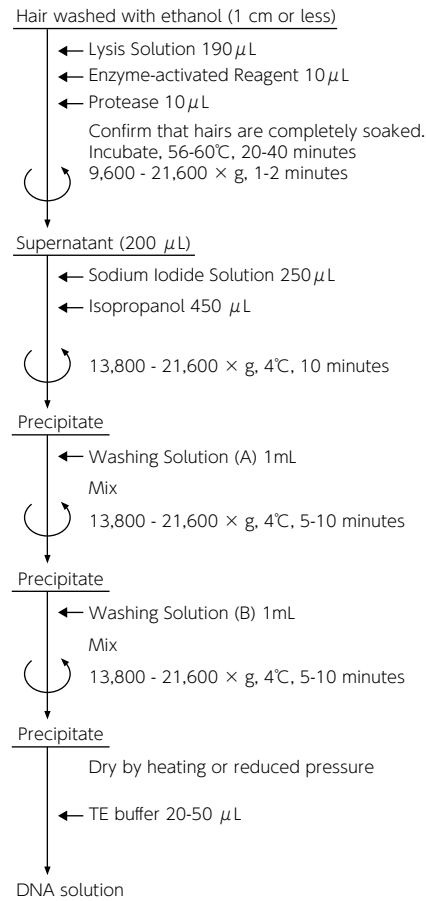
(DNA extraction from trace amount of samples such as body hairs, blood stains, saliva stains, and nails)

# DNA Extractor<sup>®</sup> FM Kit

Wako Cat. No.	Package Size
295-58501	50 tests

This kit is to extract trace amount of DNA from forensic samples such as body hairs (hairs), blood stains, saliva stains, and nails used for personal identification. Although a low yield of DNA becomes an obstacle in determination in some cases, this kit rapidly dissolves samples and efficiently collects DNA suitable for the amplification of STR (short tandem repeat) and hypervariable region of mitochondrial DNA. Also, this product uses the sodium iodide method without using organic solvents such as phenol and chloroform.

### Protocol for DNA recovery from human hairs



Operation time: 41 minutes or more  
Sample amount: hair 1 cm or less, nail 0.5 mg or less, stain 2 pieces of 5 mm  $\times$  5 mm

#### Features

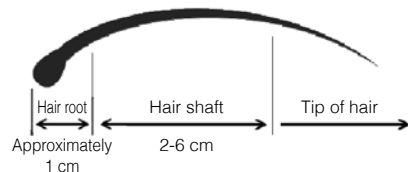
- DNA can be extracted from low-soluble and trace amount samples such as body hairs (hairs), blood stains, and nails.
- DNA can also be extracted from hair shaft.
- No solid-phase extraction reduces loss of trace amount DNA by adsorption to the carrier.
- Organic solvents such as phenol and chloroform are not required.
- DNA suitable for PCR can be obtained.

#### Kit Contents [50 tests]:

1. Lysis solution ..... 9.5 mL  $\times$  1
2. Enzyme activated reagent (EAR) ..... 80 mg  $\times$  1
3. Reconstitution solution for EAR ..... 500  $\mu$ L  $\times$  1
4. Protease ..... 10 mg  $\times$  1
5. Sodium iodide solution ..... 12.5 mL  $\times$  1
6. Washing solution (A) ..... 50 mL  $\times$  1
7. Washing solution (B) ..... 50 mL  $\times$  1

#### Sample volume

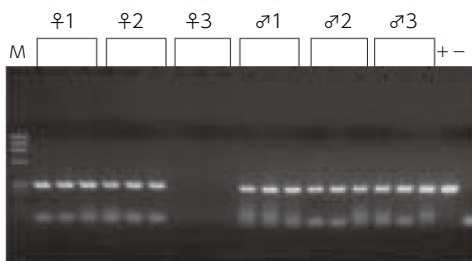
- Body hair: 1 cm or less. Two hairs or less (2 cm or less) should be used if possible due to possible inhibition of PCR reaction by melanin.
- Spots such as blood and saliva stains: Cut into a size of about 5 mm  $\times$  5 mm. Use up to 2 pieces of a 5 mm  $\times$  5 mm spot per test.
- Nail: Cut a fingernail of 0.5 mg or less (size of approximately 1 mm  $\times$  1 mm) into pieces of about 0.5 mm  $\times$  1 mm. DNA extraction will become easier with smaller pieces.



Reference Wang, L. *et al.*, *Nucleic Acids Res.*, **22**, 1774 (1994).

## DNA Extractor<sup>®</sup> FM Kit Data

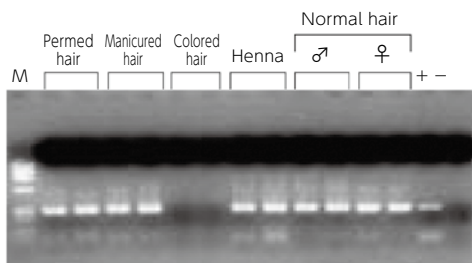
### Nucleic acid amplification reaction in hypervariable region of mitochondrial DNA (nucleotide positions 16159-16401) by using DNA extracted from hair root.



The lengths of hair shaft are all 0.5 cm, 1.0 cm, and 1.5 cm from the left of the lane for males and females.  
The same band (240 bp) was detected in all subjects except for female 3. Female 3 has dyed permed hair.

← 240bp

### Amplification of hypervariable region of mitochondrial DNA by using DNA extracted from hair dyed with different types of dyes.



The hair lengths are all 0.5 cm and 1.0 cm from the left of the lane.  
The same band (240 bp) was detected in differently dyed hairs except for hairs dyed with hair color.  
For hairs dyed with hair color, DNA itself in hairs may be affected by decomposition, etc., or the dye may have PCR inhibitory effect.

← 240bp

Description	Wako Cat. No.	Grade	Package Size
DNA Extractor <sup>®</sup> Kit	295-50201	For genetic research	50 tests
DNA Extractor <sup>®</sup> WB Kit	291-50502	For genetic research	50 tests
DNA Extractor <sup>®</sup> SP Kit	296-60501	For genetic research	50 tests
DNA Extractor <sup>®</sup> TIS Kit	296-67701	For genetic research	50 tests
DNA Extractor <sup>®</sup> FM Kit	295-58501	For forensic medicine	50 tests

Description	Wako Cat. No.	Grade/Manufacturer name	Package Size
8-OHdG Assay Preparation Reagent Set	292-67801	For genetic research	50 tests
High Sensitive 8-OHdG Check	307-07921	NIKKEN SEIL Co, Ltd.	96 tests
New 8-OHdG Check	301-06101	NIKKEN SEIL Co, Ltd.	1 set

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