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Value from Innovation

Wako

DNA extraction method using sodium iodide without phenol and chloroform

DNA Extractor® Kit Series

The sodium iodide method is introduced in USP42-NF37 "NUCLEIC ACID - BASED TECHNIQUES - APPROACHES FOR DETECTING TRACE NUCLEIC ACIDS (RESIDUAL DNA TESTING)".

FUJIFILM Wako Pure Chemical Corporation

DNA is commonly extracted from biological tissues using a method that involves phenol/chloroform treatment followed by precipitation. However, this method requires the use of phenol and chloroform, both of which are classified as hazardous substances.

DNA Extractor® Kit series uses sodium iodide, a chaotropic agent, instead of organic solvents for DNA extraction.

Sodium iodide exhibits both protein-denaturing and solubilizing effects. It releases chaotropic ions (I^-) into solution, which increase the solubility of hydrophobic molecules and weaken hydrophobic interactions. These effects contribute particularly to the denaturation and solubilization of membrane proteins, such as those found in the nuclear membrane.

Furthermore, because this protocol does not involve solid-phase extraction using silica carriers, DNA loss due to adsorption is minimized. In addition, performing all steps in a single tube helps ensure high recovery rates.

Features

- ☐ No hazardous organic solvents such as phenol or chloroform required.
- ☐ All steps performed in a single tube, minimizing DNA loss, contamination, and mechanical damage.
- ☐ No solid-phase extraction using silica carriers, preventing DNA loss from adsorption.
- ☐ Reduces oxidative and mechanical damage to DNA
- ☐ Achieves high recovery rates

List of DNA Extractor® Kit series using the sodium iodide method and residual DNA detection kit

Sample	Product Name	Features
Viral DNA from serum Residual host cell-derived DNA from biological products	**DNA Extractor® Kit**	Extract trace amount of DNA from samples
Whole blood (human, bovine, equine), cultured cells, tissues	**DNA Extractor® WB Kit**	Extract high-molecular DNA
Genomic DNA in serum/plasma	**DNA Extractor® SP Kit**	Remove blood-derived lipids
Mammalian tissues and cells	**mtDNA Extractor® CT Kit**	Extract high-purity mtDNA
Body hairs, nails, blood stains, saliva stain (forensic samples)	**DNA Extractor® FM Kit**	DNA extraction from trace amount of samples

Extraction of viral DNA from serum and residual host cell-derived DNA from biological products

DNA Extractor® Kit

Product No.	Package Size
295-50201	50 tests

This kit employs the sodium iodide method to extract viral DNA from serum and residual host cell-derived DNA from biological products. The extracted DNA is suitable for quantification by qPCR and can be used for testing and monitoring host cell DNA derived from CHO cells, *E. coli*, yeast, and other sources. When combined with Molecular Devices' Threshold systems, it also enables the quantification of total DNA, regardless of species origin.

Operation time: 1 to 1.5 hours

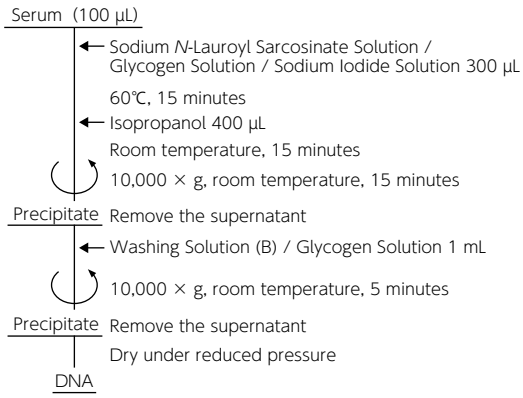
Sample amount: 100 µL/test (Clean samples such as Purified proteins or Serum),
500 µL/test (Crude samples such as Cell lysate)

Features

- ☐ Employs the sodium iodide method*.
- ☐ Efficient recovery of trace DNA (100-1,000 fg).
- ☐ All steps completed in a single tube—no tube exchange required
- ☐ Extraction completed in 60-90 minutes.
- ☐ Pre-treatment protocol available for samples with high protein content
- ☐ Recovered DNA is compatible with qPCR and Threshold Assay (Molecular Devices, LLC)

* The sodium iodide method is referenced in the U.S. Pharmacopeia 42–NF37, <509> Residual DNA Testing, as a method for residual DNA extraction.

Extraction from serum



Kit Contents [50 tests (sample 100 or 500 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. A lysis buffer containing surfactants disrupts the cytoplasm and plasma membrane, allowing selective recovery of the nucleus.
ii. Protease treatment degrades the nuclear membrane and other impurities, releasing DNA into solution.
iii. Following protein solubilization with sodium iodide, genomic DNA is selectively precipitated by the addition of isopropanol and then recovered

DNA Extractor® Kit Data

Examples of using DNA Extractor® Kit

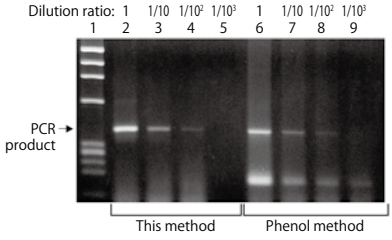
DNA Yield

	2. Culture supernatants	
Added DNA (fg)	Amount of recovered DNA (fg)	Yield (%)
0	ND	
10		
100	93	**93**
1,000	741	**74**
10,000	6,333	**63**
100,000	86,703	**87**
1,000,000	874,502	**87**

The DNA yield under each set of conditions was calculated based on a calibration curve generated from the results of the standard conditions.

Spike and recovery test of CHO cell-derived DNA

Using the kit, DNA was extracted from culture supernatants of PANC-1 cells to which 10 fg to 1 ng of CHO cell-derived DNA was added. A qPCR assay was then performed using the extracted DNA to obtain Cq values.
A qPCR was also performed using purified water to which CHO cell-derived DNA was added, without DNA extraction (standard conditions), to obtain Cq values.
DNA added to culture supernatants (100 fg to 1 ng) was extracted with high yield.



Detection of virus DNA from human serum by PCR

Human serum containing hepatitis B virus (HBV) was serially diluted, and DNA was extracted using this product and the phenol method.
HBV DNA in the extracts was amplified by PCR and analyzed by agarose gel electrophoresis.

Lane 1: Marker DNA
(ϕ X174/Hae III)

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

Related Product

qPCR (probe-based) quantification kit for *E. coli* genomic DNA

QCdetect™ Residual DNA Detection Kit for *E. coli*

Product No.	Package Size
290-85301	100 tests

This kit is used to quantify *Escherichia coli* genomic DNA (gDNA) by probe-based qPCR.
It is designed to extract residual host cell DNA during quality control and process development of biopharmaceuticals.

Features

- ☐ Limit of detection : ≧ 0.03 pg/test
- ☐ Limit of quantification : ≧ 0.3 pg/test
- ☐ Calibration curve with high linearity
- ☐ High reproducibility with low inter-assay variability
- ☐ Convenient operation using a pre-mixed buffer
- ☐ Low susceptibility to interference from sample impurities
- ☐ Internal Control included

Detection Wavelength

- ☐ *E. coli* gDNA 520 nm (Example : FAM)
- ☐ Internal Control 555 nm (Example : HEX)
  ＊ The Internal Control is a synthetic DNA sequence that does not occur in nature.

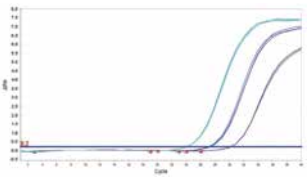
QCdetect™ Residual DNA Detection Kit for *E. coli* Data

Example of application: protein-rich sample prepared using DNA Extractor® Kit

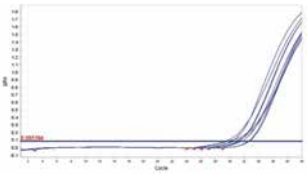
Genomic DNA from *E. coli* was spiked into a sample containing 75 mg/mL human plasma-derived γ-globulin at concentrations of 0.1, 1 and 10 ng/mL. DNA was subsequently extracted using the DNA Extractor® Kit.

DNA loading amount	0.1 ng/mL	1 ng/mL	10 ng/mL
Amount of DNA added to the detection system (PCR reaction)	1 pg	10 pg	100 pg
Recovery rate (average)	96.9%	85.7%	86.9%
SD	0.013	0.67	1.4
CV%	1.3%	7.8%	1.6%

E. coli-derived genomic DNA



Internal Control



Even from samples containing high levels of protein, *E. coli* gDNA was efficiently recovered at high yield.

qPCR (Probe-Based) Kit for quantification of CHO cell genomic DNA

QCdetect™ Residual DNA Detection Kit for CHO cells

Product No.	Package Size
294-85201	100 tests

This product is a probe-based qPCR kit for quantifying genomic DNA (gDNA) derived from CHO cells.
It is designed for residual DNA testing in antibody therapeutics and during their manufacturing processes.

Features

- ☐ Limit of detection : ≧ 0.0003 pg/test
- ☐ Limit of quantification : ≧ 0.003 pg/test
- ☐ Calibration curve with high linearity
- ☐ High reproducibility with low inter-assay variability
- ☐ Convenient operation using a pre-mixed buffer
- ☐ Low susceptibility to interference from sample impurities
- ☐ Internal Control included

Detection Wavelength

- ☐ CHO gDNA 520 nm (Example : FAM)
- ☐ Internal Control 554 nm (Example : HEX)
  ＊ The Internal Control is a synthetic DNA sequence that does not occur in nature.

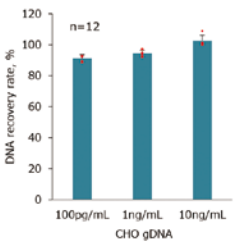
QCdetect™ Residual DNA Detection Kit for CHO cells Data

Example of Application: protein-rich sample prepared using DNA Extractor® Kit

CHO gDNA was spiked into a sample containing a high concentration of γ-globulin, and DNA was extracted using the DNA Extractor® Kit.

Composition of the Sample
- 20 mg/mL γ-globulin
- 3% Mannitol
- 2% Sucrose
- 10mM L-Arginine
- 0.01% Tween20

Concentration of spiked CHO gDNA
- 10 ng/mL
- 1 ng/mL
- 100 pg/mL



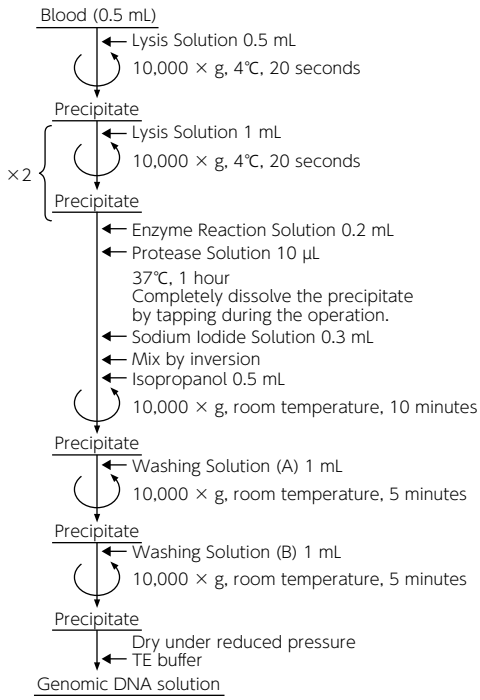
Even from samples containing high levels of protein, CHO gDNA was efficiently recovered at high yield.

Genomic DNA extraction from whole blood (human, bovine, and equine), cultured cells, and tissues

DNA Extractor® WB Kit

Product No.	Package Size
291-50502	50 tests

High-molecular-weight genomic DNA is essential for human genome analysis. Although various DNA extraction methods are currently in use, most involve organic solvents such as phenol. These substances are hazardous, and the procedures tend to be labor-intensive and time-consuming. This kit is specifically designed for extracting genomic DNA from human whole blood.
It enables high-yield, high-purity DNA extraction in a short time using a single tube, without the use of hazardous solvents such as phenol or chloroform.



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

Features

- ☐ High-molecular-weight DNA can be recovered with yields exceeding 90%
- ☐ Entire process completed in approximately 1.5 hours in a single tube
- ☐ No need for organic solvents such as phenol or chloroform
- ☐ Suitable for DNA extraction from human whole blood, bovine/equine blood, cultured cells, and tissues
- ☐ Simpler than the phenol/chloroform extraction method; suitable for processing multiple samples
- ☐ Single-tube protocol minimizes mechanical shearing and damage to genomic DNA

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

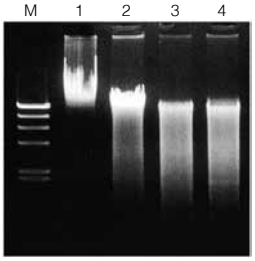
1. Lysis Solution ..... 65 mL × 2
2. Enzyme Reaction Solution ..... 10 mL × 1
3. Sodium Iodide Solution ..... 15 mL × 1
4. Washing Solution (A) ..... 50 mL × 1
5. Washing Solution (B) ..... 50 mL × 1
6. Protease ..... 10 mg × 1

Principle of DNA extraction

i. A lysis buffer containing surfactants disrupts the cytoplasm and plasma membrane, allowing selective recovery of the nucleus.
ii. Protease treatment degrades the nuclear membrane and other impurities, releasing DNA into solution.
iii. Following protein solubilization with sodium iodide, genomic DNA is selectively precipitated by the addition of isopropanol and then recovered.

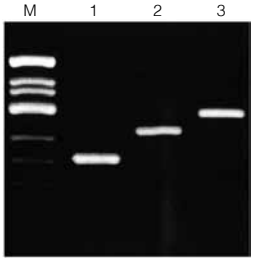
DNA Extractor® WB Kit Data

Examples of using DNA Extractor® WB Kit



M: Marker DNA (λ /Hind III)
Lane 1: Undigested human genomic DNA
Lane 2: Genomic DNA/BamH I
Lane 3: Genomic DNA/EcoR I
Lane 4: Genomic DNA/Hind III

Agarose gel electrophoresis pattern of genomic DNA extracted from human whole blood using this kit, after digestion with various restriction enzymes



M: Marker DNA (ϕ X174/Hinf I)
Lane 1: 262 bp
Lane 2: 345 bp
Lane 3: 408 bp

Genomic DNA extracted from human whole blood using this kit was amplified by PCR and analyzed by electrophoresis. Various sizes of human β-globin gene fragments were amplified by PCR using 1 µg of genomic DNA as template.

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

Genomic DNA extraction from bovine/equine whole blood

By slightly modifying the standard protocol, high-purity genomic DNA can be efficiently extracted from the whole blood of equine and bovine.
For equine blood, protease digestion is performed for 2 hours.
For bovine blood, the washing step with lysis buffer is repeated four times, followed by a 4-hour protease digestion.
The total processing time is approximately 3 hours for equine blood and 5 hours for bovine blood.
Blood samples containing EDTA-2Na or heparin as anticoagulants were used.

Electrophoretic image of genomic DNA extracted from bovine/equine

Lane 1: λ /Hind III
Lane 2: Equine blood (EDTA·2Na)
Lane 3: Equine blood (Heparin)
Lane 4: Bovine blood (EDTA·2Na)

3 µL of 100 µL extracted DNA solution was loaded on a 1% agarose gel and DNA was separated by electrophoresis.

Operation procedure

DNA extraction from equine blood
Perform protease digestion for 2 hours or until the nuclear pellet is no longer visible. Follow the standard protocol for all other steps.

DNA extraction from bovine blood
In the step where nuclei are selectively isolated using lysis buffer, two washes as per the standard protocol are often insufficient to remove impurities such as hemoglobin. Repeat this step 4 to 5 times, or until the red pellet becomes nearly colorless.
The pellet is very fragile after centrifugation, so handle with care during decanting.
Protease digestion should then be performed for approximately 4 hours.

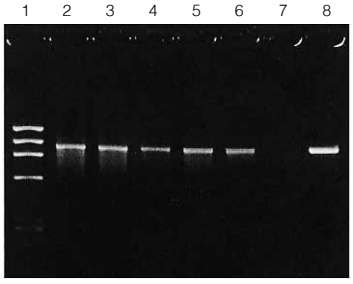
Yield and purity of genomic DNA extracted from bovine/equine

Sample	DNA yield (µg/mL blood)	A_{260}/A_{280}
Equine (EDTA·2Na)	56.62	1.91
Equine (Heparin)	48.34	1.88
Bovine (EDTA·2Na)	22.04	1.86

Notes) Each equine blood sample was collected from different equines.

DNA extraction from rat tissues

By adding two or three simple steps to the standard protocol, genomic DNA can be extracted from tissues in approximately 2 hours.
The resulting DNA contains minimal RNA and protein impurities, and can be used directly for restriction enzyme digestion, PCR, Southern blotting, and DNA fingerprinting.



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

Examples of rat gene detection by PCR

PCR amplification of a 983-bp fragment of the rat GAPDH gene was performed using DNA extracted from various rat tissues as the template.

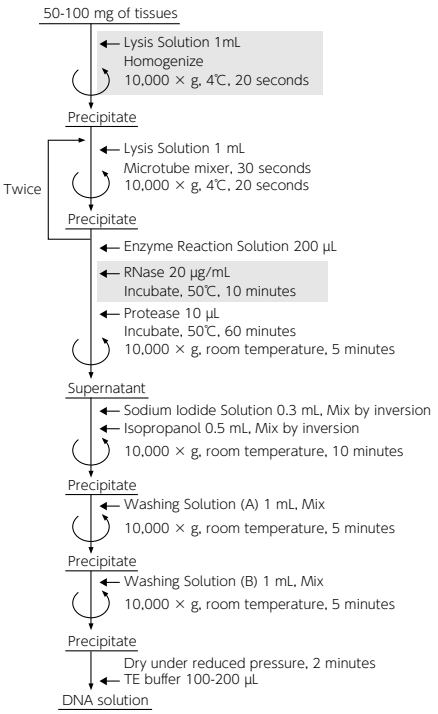
Lane 1: Marker DNA
(ϕ X174/Hae III)
Lane 2: Stomach
Lane 3: Intestine
Lane 4: Liver
Lane 5: Pancreas
Lane 6: Skeletal muscles
Lane 7: Negative control
Lane 8: Positive control

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

Tissues	DNA yield (µg/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. What are the differences from DNA Extractor® Kit (Product No. 295-50201)?
The purpose of use is different. DNA Extractor® Kit is for the extraction of a slight amount of viral DNA (DNA virus such as hepatitis B virus) in serum and a slight amount of fungus-derived DNA in biological products, while DNA Extractor® WB Kit specifically extracts genomic DNA from whole blood and cultured cells.

Q4. 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® CT Kit (Product No. 291-55301) is recommended.

Q5. 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.

Q6. 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.

Q7. 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.

Q8. 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.

Q9. Can DNA be extracted from tissues with this kit?
Yes. DNA can be extracted by fragmenting tissues by homogenization.

Q10. What is the initial cell number when extracting DNA from cultured cells?
10^3 to 10^6 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.

Q11. What should be done without a microtube mixer?
Mix the sample by inversion for about 1 to 2 minutes.

Q12. We want to suspend the experiment.
Store the precipitate separated by centrifugation with the addition of lysis solution at -20°C.

Q13. We do not have a refrigerated centrifuge.
Extraction can be performed with a tabletop centrifuge. In that case, perform the operation quickly.

Q14. How should protease be stored after prepared?
It is stable at -20°C for 6 months.

Q15. 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.

Q16. 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.

Q17. 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.

Q18. The A_{260}/A_{280} 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).

Q19. Is there any reference literature?
See the following reference: Wang, L., Hirayasu, K., Ishizawa, M. and Kobayashi, Y.: *Nucl. Acids Res.*, **22**, 1774 (1994).

Genomic DNA extraction from serum and plasma

DNA Extractor® SP Kit

Product No.	Package Size
296-60501	50 tests

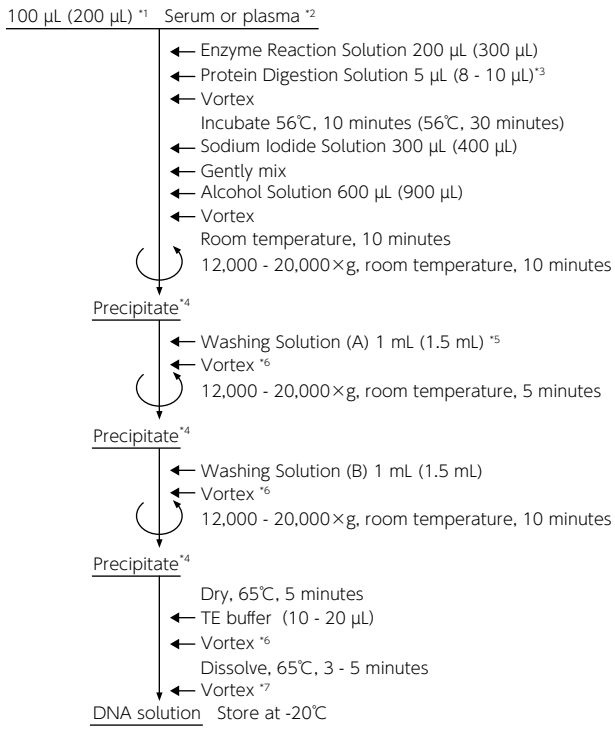
The DNA Extractor® SP Kit is an improved version of the DNA Extractor® Kit, specifically designed for extracting DNA fragments from serum or plasma. With its high DNA recovery rate, it serves as an effective preprocessing reagent for the sensitive detection and analysis of target genes. The kit employs the sodium iodide method and eliminates the need for hazardous organic solvents such as phenol or chloroform.

Operation time: 1 hour or more
Sample amount: 100 µL/test

Features

- ☐ Enables high DNA recovery from small volumes (100 µL) of serum or plasma
- ☐ Utilizes the highly efficient sodium iodide method (near 100% recovery)
- ☐ No phenol, chloroform, or other organic solvents required
- ☐ Avoids solid-phase extraction using silica carriers, ensuring high DNA recovery
- ☐ Provides DNA suitable for PCR
- ☐ Effectively removes blood-derived lipids
- ☐ Entire procedure completed in a single tube

Methods



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

1. Enzyme Reaction Solution ····· 10 mL × 1
2. Protein Digestion Solution ····· 250 µL × 1
3. Sodium Iodide Solution ····· 15 mL × 1
4. Alcohol Solution ····· 30 mL × 1
5. Washing Solution (A) ····· 50 mL × 1
6. Washing Solution (B) ····· 50 mL × 1

Principle of DNA extraction

i. Proteases degrade proteins derived from serum or plasma.

ii. Sodium iodide solubilizes proteins and lipids derived from serum or plasma; these impurities are then removed using the alcohol solution provided in the kit, and DNA is precipitated.

∗1 The protocol starting with 200 µL 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

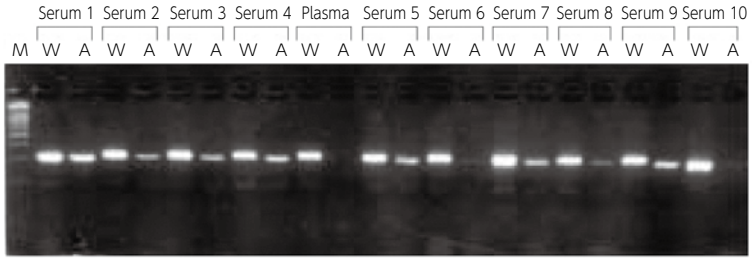
1) Ishizawa, M. *et al., Nucleic Acids Res.*, **22**, 1774 (1994).
2) Sozzi, G. *et al., Clin. Cancer Res.*, **5**, 2689 (1999).
3) Silva, J. M. *et al., Cancer Res.*, **59**, 3251 (1999).
4) Shao, Z. M. *et al., Clin. Cancer Res.*, **7**, 2222 (2001).

DNA Extractor® SP Kit Data

Examples of using DNA Extractor® SP Kit

Amplification of the p53 exon 5 region from DNA extracted from human serum and plasma

DNA was extracted from 10 human serum samples and 1 plasma sample using this kit.
The extracted DNA was dissolved in 20 µL of TE buffer (pH 8.0), and 5 µL of each sample was subjected to PCR amplification of the 308-bp p53 exon 5 region. As a control, a DNA extraction kit from Company A employing silica-based centrifugal filtration was used for comparison.



DNA extraction from 1 - 5 mL samples using the DNA Extractor® SP Kit

When DNA was dissolved in TE buffer, insoluble precipitates remained, and background absorbance at 320 nm was observed, indicating lower purity compared to standard extractions from 100 - 200 µL samples. Nevertheless, the DNA was still suitable for PCR amplification.

Method for treating 1 mL serum (plasma) sample

1 mL sample/15 mL centrifuge tube
- ← 2 mL Enzyme Reaction Solution
- ← 50 µL Protein Digestion Solution
  56℃, 30 minutes
- ← 3 mL Sodium Iodide Solution
- ← 6 mL Alcohol Solution
  Room temperature, 15 minutes
- 10,000 rpm, 20 minutes

Precipitate
- ← 10 mL Washing Solution (A)
- 10,000 rpm, 10 minutes

Precipitate
- ← 10 mL Washing Solution (B)
- 10,000 rpm, 10 minutes

Precipitate
(Dissolve in an appropriate amount of TE, etc.)

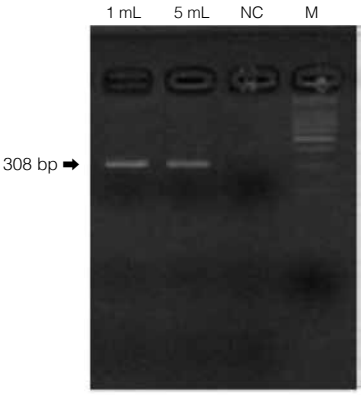
Method for treating 5 mL serum (plasma) sample

5 mL sample/50 mL centrifuge tube
- ← 7.5 mL Enzyme Reaction Solution
- ← 250 µL Protein Digestion Solution
  56℃, 30 minutes
- ← 10 mL Sodium Iodide Solution
- ← 22.5 mL Alcohol Solution
  Room temperature, 15 minutes
- 10,000 rpm, 20 minutes

Precipitate
- ← 38 mL Washing Solution (A)
- 10,000 rpm, 10 minutes

Precipitate
- ← 38 mL Washing Solution (B)
- 10,000 rpm, 10 minutes

Precipitate
(Dissolve in an appropriate amount of TE, etc.)



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.

Mitochondrial DNA extraction kit for tissues, cells, and compound feed

mtDNA Extractor® CT Kit

Product No.	Package Size
291-55301	25 tests

Preparing highly pure mtDNA in the presence of abundant genomic DNA is typically labor-intensive and time-consuming. mtDNA Extractor® CT Kit enables rapid and simple extraction of mitochondrial DNA (mtDNA) from mammalian tissues and cells.It can also be used to extract mtDNA from compound feed.

Methods

Tissue (50 mg) or muscle tissue (250 mg)
← Buffer for Homogenate 1 mL
Homogenize tissue at 1,000 rpm using a homogenizer on ice
Homogenate
1,000×g, 4℃, 1 min
Supernatant
10,000×g, 4℃, 10 min
Precipitate
← DNA Extraction Solution Ⅰ 50 µL
← DNA Extraction Solution Ⅱ 100 µL*
*: DNA Extraction Solution Ⅱ are prepared by mixing 50 µL of DNA Extraction Solution Ⅱ (A) and (B) just before use.
The prepared solution is stable for 3 weeks at 2~10℃.
← Cold DNA Extraction Solution Ⅲ 75 µL
Leave it for 5 min. on ice
12,000×g, 4℃, 5 min
Supernatant
← Sodium Iodide Solution 300 µL
← Mix well
← Isopropanol 500 µL
← Mix well
12,000×g, room temperature, 10 min
Precipitate
← Washing Solution 1 mL
Vortex
12,000×g, room temperature, 5 min
Precipitate
Dry in vaccuo
← TE buffer (pH 8.0)
mtDNA Solution

Features

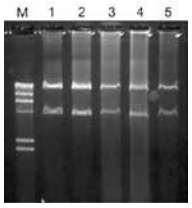
- ☐ mtDNA in amounts detectable by agarose gel electrophoresis can be extracted from 50-250 mg of tissue.
- ☐ The extracted mtDNA is suitable for PCR and restriction enzyme digestion.
- ☐ Frozen tissues can also be used for extraction.
- ☐ The protocol does not require organic solvents such as phenol or chloroform.

Kit Contents [25 tests]:

1. Buffer for Homogenate ····· 25 mL × 1
2. DNA Extraction Solution I ····· 1.3 mL × 1
3. DNA Extraction Solution II (A) ····· 1.3 mL × 1
4. DNA Extraction Solution II (B) ····· 1.3 mL × 1
5. DNA Extraction Solution III ····· 1.9 mL × 1
6. Sodium Iodide Solution ····· 7.5 mL × 1
7. Washing Solution ····· 50 mL × 1

mtDNA Extractor® CT Kit Data

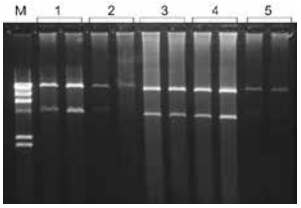
Example of mtDNA Extractor® CT Kit



M: λ /Hind III
Lane1: Brain
Lane2: Heart
Lane3: Liver
Lane4: Kidney
Lane5: Skeletal muscle

SYBR® Green I
staining 1% Agarose

Half of the mtDNA extracted from fresh mouse tissue was digested with Pst I and analyzed by agarose gel electrophoresis.



M: λ /Hind III
Lane1: Brain
Lane2: Heart
Lane3: Liver
Lane4: Kidney
Lane5: Skeletal muscle

SYBR® Green I
staining 1% Agarose

Half of the mtDNA extracted from frozen mouse tissue was digested with Pst I and analyzed by agarose gel electrophoresis.

Mitochondrial DNA extracted using this kit is present in small amounts and cannot be detected on a gel without staining with a sensitive dye such as GelRed™. It is not detectable using ethidium bromide staining.

SYBR® is a registered trademark of Thermo Fisher Scientific.

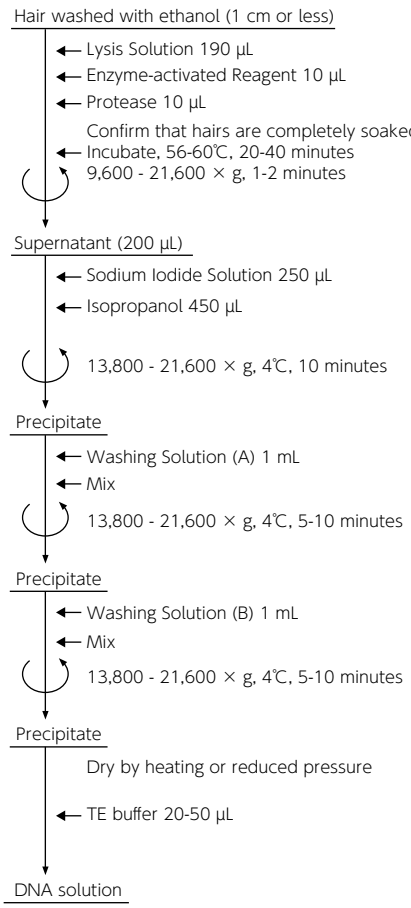
DNA extraction from forensic samples (body hairs, blood stains, saliva stains, and nails)

DNA Extractor® FM Kit

Product No.	Package Size
295-58501	50 tests

The DNA Extractor® FM Kit is designed to extract trace amounts of DNA from forensic samples, such as hair, blood stains, saliva stains, and nails, which are commonly used for identifying individuals.
Low DNA recovery from such samples often poses a challenge in forensic analysis; however, this kit enables rapid sample dissolution and efficient recovery of DNA suitable for amplification of STRs and mitochondrial hypervariable regions. It employs the sodium iodide method and eliminates the need for hazardous organic solvents such as phenol or chloroform.

Protocol for DNA recovery from human hairs



Sample amount: hair 1 cm or less, nail 0.5 mg or less, blood or saliva stains 2 pieces of 5 mm × 5 mm

Features

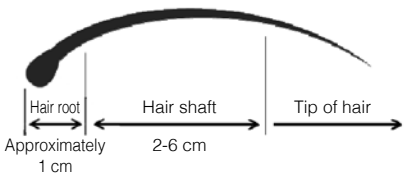
- ☐ Enables DNA extraction from trace forensic samples such as hair, blood stains, and nails
- ☐ No solid-phase extraction, minimizing loss of trace DNA due to adsorption to carriers
- ☐ No hazardous solvents such as phenol or chloroform required
- ☐ Yields DNA suitable for PCR

Kit Contents [50 tests]:

1. Lysis Solution ····· 9.5 mL × 1
2. Enzyme Activated Reagent (EAR) ····· 80 mg × 1
3. Reconstitution Solution for EAR ····· 500 µL × 1
4. Protease ····· 10 mg × 1
5. Sodium Iodide Solution ····· 12.5 mL × 1
6. Washing Solution (A) ····· 50 mL × 1
7. Washing Solution (B) ····· 50 mL × 1

Sample volume

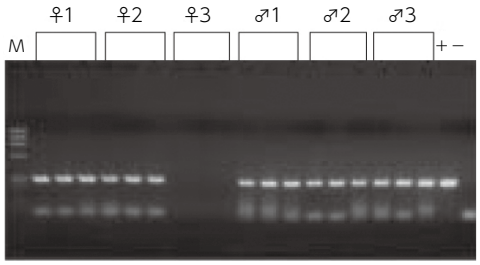
- Hair: Less than 1 cm in length. Since melanin may inhibit amplification, it is recommended to use no more than two hairs (total length up to 2 cm).
- Blood or saliva stains: Cut to approximately 5 mm x 5 mm. Up to two pieces of this size can be processed per test.
- Nails: Cut into pieces approximately 0.5 mm x 1 mm in size, keeping the total amount under 0.5 mg (equivalent to a ~1 mm square). Finer cutting improves DNA extraction efficiency.



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

DNA Extractor® FM Kit Data

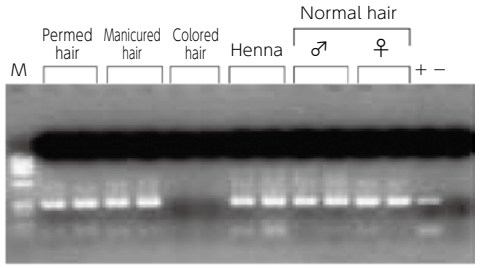
PCR amplification of the mitochondrial hypervariable region (nucleotide positions 16159–16401) using DNA extracted from hair shafts



Hair shaft lengths were 0.5 cm, 1.0 cm, and 1.5 cm (left to right in each lane).
A 240-bp band was detected in all samples except that of Female 3, whose hair was dyed and permed.

⬅ 240 bp

Amplification of the mitochondrial hypervariable region using DNA extracted from hair shafts treated with different dyeing methods



Hair shaft lengths were 0.5 cm and 1.0 cm (left to right in each lane).
A 240-bp band was detected in all samples except those treated with hair color.
The failure to amplify DNA from hair colored with permanent dye may be due either to DNA degradation within the hair or to PCR inhibition by the dye itself.

⬅ 240 bp

Product Name	Product No.	Grade	Package Size
DNA Extractor® Kit	295-50201	For Genetic Research	50 tests
DNA Extractor® WB Kit	291-50502	For Whole Blood DNA Extraction	50 tests
DNA Extractor® SP Kit	296-60501	For Genetic Research	50 tests
mtDNA Extractor® CT Kit	291-55301	For Cell and Tissue mtDNA Extraction	25 tests
DNA Extractor® FM Kit	295-58501	–	50 tests

Related Products

Product Name	Product No.	Grade	Package Size
QCdetect™ Residual DNA Detection Kit for *E.coli*	290-85301	For Genetic Research	100 tests
QCdetect™ Residual DNA Detection Kit for CHO cells	294-85201	For Genetic Research	100 tests

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