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Near your partner

You can find your partners, iNtRON Distributor in Page.

Improved performance

High quality and quantity of DNA recovery

Effective

Effective control of inhibitors and contaminants for accurate down stream applications. And the freeze-dried formulated enzyme has been improved DNA extraction stability

High Quality

Isolated high-quality DNA is suitable for many gene expression profiling techniques :

- ✓ Conventional PCR
- ✓ Quantitative PCR
- ✓ Genotyping such as STR analysis

Stage-up

No need various DNA Extraction kit – vast applicability. The Kit is suitable to use various kinds of biological samples. Advanced GxN technology for rapid and efficient purification of DNA without ethanol precipitation.

DNA Extraction | January, 2012 (1st Edition)

ISO 9001 | ISO 14001

Instruction manual

i-genomic Clinic DNA Extraction Mini Kit

The Instruction Manual for purification of genomic and mitochondrial DNA from Blood, serum, Biological fluid, fixed tissues, Cigarette butts, swabs, dried blood spots, stains, hair (hair root / hair shaft), nail clippings, and chewing gum using silica membrane.

REF 17384 Σ 50

RUO

RT

Product info.

iNtRON Biotechnology

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◈ Molecular Reagent

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DESCRIPTION

- The i-genomic Clinic DNA Extraction Mini Kit uses well-established technology for purification of genomic and mitochondrial DNA from various samples for clinical studies. The kit provides a fast and easy way to purify DNA from not only blood, serum, Biological fluid, and fixed tissues but also small amount sample like forensic samples such as cigarette butts, swabs, dried blood spots, stains, hair (hair root / hair shaft), nail clippings, and chewing gum. The enhanced lysis efficiency and binding capacity will help to DNA extraction from various samples as maximized efficiency.
- The procedure is designed to ensure that there is no sample-to-sample cross-contamination and allows safe handling of potentially infectious samples. After sample lysis, the simple procedure, which is highly suited for simultaneous processing of multiple samples, yields pure DNA in less than 30 minutes. DNA is eluted in Buffer CE or water and is immediately ready for use in amplification reactions or for storage at -20°C. Purified DNA is free of proteins, nucleases and other impurities.
- The kit provides 10 kinds of protocols. You can also extract genomic DNAs from various samples by selecting an appropriate protocol. If you need some more information in selecting a protocol, please do not hesitate to contact our Technical Assistance Team.

CHARACTERISTICS

- High quality and quantity of DNA recovery
- Effective control of inhibitors and contaminants for accurate down stream applications. And the freeze-dried formulated enzyme has been improved DNA extraction stability.
- Isolated high-quality DNA is suitable for many gene expression profiling techniques :
  - ✓ Conventional PCR
  - ✓ Quantitative PCR
  - ✓ Genotyping such as STR analysis
- Advanced GxN technology for rapid and efficient purification of DNA without ethanol precipitation.
- No need various DNA Extraction kit – vast applicability. The Kit is suitable to use various kinds of biological samples.

KIT CONTENTS

Label	Description	Contain
Buffer CCL	Pre-Lysis Buffer	25 ml
Buffer CBL	Lysis Buffer	25 ml
Buffer CWA	Washing Buffer A	40 ml
Buffer CWB (Concentrate) [1]	Washing Buffer B	10 ml
Buffer CE [2]	Elution Buffer	20 ml
Spin Columns (Violet color O-ring)	Inserted into a collection tubes. (2.0ml tubes)	50 columns
Collection Tubes (2.0 ml tubes)	Additionally supplied.	100 tubes
Binding Carrier[3]	Enhance binding of DNA (store at 4°C upon arrival)	0.45 ml
RNase A (Lyophilized powder)[4]	Dissolve in 0.3ml of DW	3 mg
Proteinase K (Lyophilized powder)[4]	Dissolve in 1.1ml of DW	22 mg

1. This buffer contains chaotropic salt.

2. *Before use, add 40ml of absolute EtOH to the washing buffer.*

3. The Binding Carrier should be stored at 4°C upon arrival.

4 The lyophilized RNase A and Proteinase K can be stored at room temperature (15–25°C) until the kit expiration date without affecting performance. The lyophilized RNase A and Proteinase K can only be dissolved in D.W.; dissolved RNase A and Proteinase K should be immediately stored at -20°C. These solutions are stable at -20°C for up to 24 months and 20 times frozen-thawing until the kit expiration date.

STORAGE

i-genomic Clinic DNA Extraction Mini Kit should be stored dry at room temperature (15–25°C). Under these conditions, i-genomic Clinic DNA Extraction Mini Kit can be stored for up to 24 months without showing any reduction in performance and quality. The lyophilized RNase A and Proteinase K can be stored at room temperature (15–25°C) until the kit expiration date without affecting performance. The lyophilized RNase A and Proteinase K can only be dissolved in D.W.; dissolved RNase A and Proteinase K should be immediately stored at -20°C. These solutions are stable at -20°C for up to 24 months and 20 times frozen-thawing until the kit expiration date.

◈ Down stream applications – Genetic identification

After extracting DNA from deeply contaminated cigarette butt by using i-genomic Clinic DNA Mini Kit, the extracted DNA was analyzed a short tandem repeat (STR) multiplex assay that amplifies 15 tetranucleotide repeat loci and the Amelogenin gender determining marker in a single PCR amplification.

The result was that DNA from contaminated butt had sufficient level to DNA identification. It means that i-genomic Clinic DNA Mini Kit is able to derive high yield and efficiently remove inhibitors from micro-amount mutilated samples.

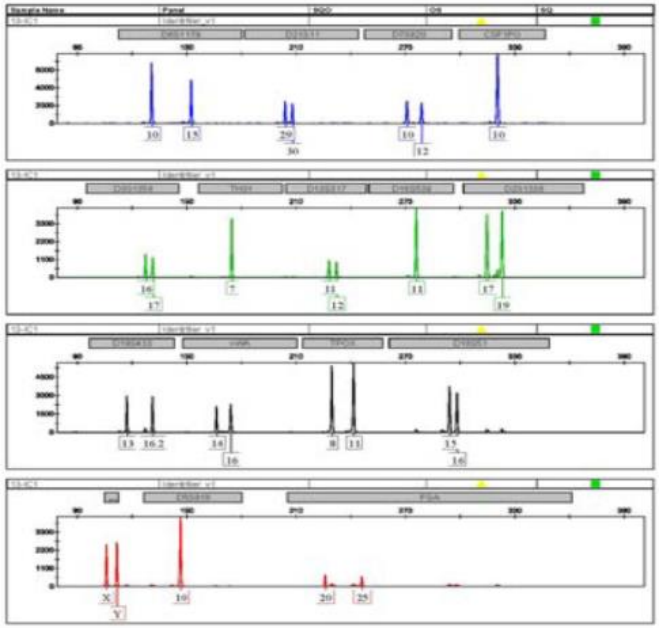


Fig 2. DNA Profiling Test

In order to estimate the possibility of down stream application which need extra high quality and sensitivity such as DNA profiling assay, DNA sample was prepared from deeply contaminated cigarette butt ½ pieces using i-genomic Clinic DNA Extraction Kit then STR fragments were amplified using AmpFLSTR Identifiler PCR Amplification Kit (Applied biosystems)

EXPERIMENTAL INFORMATION

◈ Effective DNA extraction from Clinical Samples

gDNA was extracted from various clinical samples (Biological swabs) by using i-genomic Clinic DNA Mini Kit and extracted gDNA was tested if it showed positive or negative for *Chlamydia trachomatis* and *Neisseria gonorrhoeae* (STD bacteria). The positive detection rate was equivalent to the detection rate of competitor's specialized product to micro sample amount.

Table 4. Evaluation of clinical application of i-genomic Clinic DNA Mini Kit.

	CT positive (n=10)	NG Positive (n=2)	CT/NG negative (n=3)	detection efficiency
iNtRON	10/10	2/2	0/2	100%
Supplier A	7/10	0/2	0/2	58%
Supplier B	10/10	2/2	0/2	100%

◈ Comparative Test of DNA Recovery from Forensic Samples with **Supplier's**

Genomic DNA was extracted from various samples (Finger print swab, gum, nail, hair, blood swab and etc.) by using i-genomic Clinic DNA Mini Kit and comparison test was implemented. Extracted DNA is used to RNase P gene specific primer and probe to run real time PCR. It was shown that the Ct value of i-genomic Clinic DNA Mini Kit was lower than that of competitors (It means higher yield). The vertical axis of the graph indicates inverse value of Log10 (Ct value), which means relative yield.

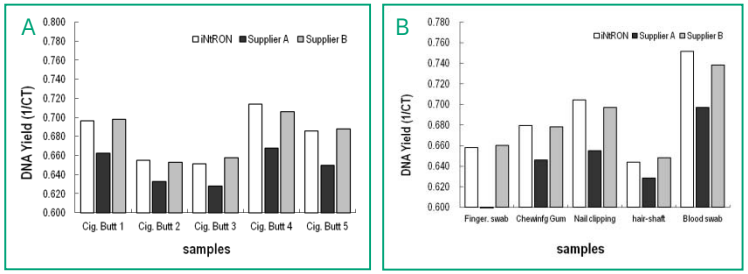


Table 4. Evaluation of clinical application of i-genomic Clinic DNA Mini Kit.

Panel A : Cigarette butt sample, Panel B : other forensic samples (Fingerprint swab, chewing gum, nail clipping, hair shaft, blood swab – from left)

CONSIDERATION BEFORE USE

- ⚠ Lyophilized RNase A : Dissolve the RNase A in 0.3 ml of pure D.W. to each vial. The lyophilized RNase A can be stored at room temperature (15–25°C) until the expiration date without affecting performance. The lyophilized RNase A can only be dissolved in D.W.; dissolved RNase A should be immediately stored at -20℃. The RNase A solution is stable at -20°C for up to 24 months and 20 times frozen-thawing until the kit expiration date.
- ⚠ Lyophilized Proteinase K : Dissolve the Proteinase K in 1.1 ml of pure D.W. to each vial. The lyophilized Proteinase K can be stored at room temperature (15–25°C) until the expiration date without affecting performance. The lyophilized Proteinase K can only be dissolved in D.W.; dissolved Proteinase K should be immediately stored at -20℃. The Proteinase K solution is stable at -20°C for up to 24 months and 20 times frozen-thawing until the kit expiration date.
- Buffer CWB
  Buffer CWB is supplied as concentrate. Before using for the first time, be sure to add 40 ml of absolute ethanol (96 - 100%) to obtain a working solution.
- Preheat a water bath or heating block
- Equilibrate samples to room temperature (15-25 °C).
- Equilibrate Buffer CE or distilled water for elution to room temperature.
- If Buffer CCL or Buffer CBL contains precipitates, dissolve by heating to 70°C with gentle agitation.
- Centrifugation
  All centrifugation steps are carried out at RT (15 - 25°C)

SAFETY INFORMATION

All chemicals should be considered as potentially hazardous. When working with chemicals, always wear a suitable lab coat and disposable glove. Some buffer contain the chaotropic salt which may be an irritant and carcinogen, so appropriate safety apparel such as gloves and eye protection should be worn. If a spill of the buffers occurs, clean with a suitable laboratory detergent and water.
If the liquid spill contains potentially infectious agents, clean the affected area first with laboratory detergent and water, then with a suitable laboratory disinfectant. Only persons trained in laboratory techniques and familiar with the principles of good laboratory practice should handle these products.

⚠ DO NOT add bleach or acidic solutions directly to the sample preparation waste.

ADDITIONAL REQUIRED EQUIPMENT

The i-genomic Clinic DNA Extraction Mini Kit provides almost all reagents for extracting DNA. However, you should prepare some equipments and reagents as follows for a fast and easy extraction. When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles.

Common equipment and reagents

- Ethanol (96 - 100%)
- Micro-pipettes and pipette tips
- Microcentrifuge
- Water bath or heating block
- Vortex mixer
- Micro centrifuge tubes (1.5 ml)
- 1M DTT
- Other general lab equipment
- Ice
- 1X PBS Buffer
- Xylene Solution (for paraffin block)

QUALITY CONTROL

- In accordance with iNtRON's ISO-certified Total Quality Management System, each lot of i-genomic Clinic DNA Extraction Mini Kit is tested against predetermined specifications to ensure consistent product quality.
- i-genomic column control : The DNA binding capacity was tested by determining the recovery with 10 ~ 15 µg of genomic DNA from 1 x 10^6 cultivated cells.
- RNase A / Proteinase K : In case of RNase A, the activity was determined 20K ~ 25K unit per mg of protein using tolura yeast RNA hydration test. Also, in case of Proteinase K, the activity was determined from cleavage of the substrate releasing p-nitroaniline which can be measured spectrophotometrically at 410nm.
- Binding Carrier : The Binding Carrier Solution was tested its enhancing efficiency of micro-amount DNA extraction from 100 cells as start material.
- Buffer control : Conductivity and pH of buffers were tested and found to be within the pre-determinated ranges descrbed below.

Buffer	Conductivity	pH
Buffer CCL	13.5 ~ 15.5 mS/cm	7.6 ~ 8.3
Buffer CBL	120 ~ 140 mS/cm	6.9 ~ 7.6
Buffer CWA	28 ~ 36 mS/cm	6.9 ~ 7.7
Buffer CWB	10 ~ 12 mS/cm	7.4 ~ 8.0
Buffer CE	550 ~ 700 µS/cm	7.4 ~ 8.0

◈ Storage, quantification, and determination of quality and yield of gDNA

Storage of DNA

For long-term storage, DNA should be dissolved in TE buffer or Buffer CE, and stored at –20°C. DNA stored in water is subject to acid hydrolysis. Any contaminants in the DNA solution may lead to DNA degradation. Avoid repeated freeze-thawing as this will lead to precipitates. We recommend storing genomic DNA samples in aliquots.

Quantification of DNA

DNA concentration can be determined by measuring the absorbance at 260 nm (A260) in a spectrophotometer using a quartz cuvette. For the greatest accuracy, readings should be between 0.1 and 1.0.

An absorbance of 1 unit at 260 nm corresponds to 50 µg genomic DNA per ml (A260 =1 ⇒ 50 µg/ml). This relation is valid only for measurements made at neutral ~ slighly alkaline pH, therefore, samples should be diluted in a low-salt buffer with slightly alkaline buffer (e.g., 10 mM Tris·Cl, pH 7.5~8.0)

Note : If you will use more than one cuvette to measure multiple samples, the cuvettes must be matched.

Note : Spectrophotometric measurements do not differentiate between DNA and RNA, so RNA contamination can lead to overestimation of DNA concentration.

Note : Phenol has an absorbance maximum of 270–275 nm, which is close to that of DNA. Phenol contamination mimics both higher yields and higher purity, because of an upward shift in the A260 value.

Purity of DNA

The ratio of the readings at 260 nm and 280 nm (A260/ A280) provides an estimate of DNA purity with respect to contaminants that absorb UV light, such as protein. The A260/ A280 ratio is influenced considerably by pH. Since water is not buffered, the pH and the resulting A260/ A280 ratio can vary greatly. Lower pH results in a lower A260/ A280 ratio and reduced sensitivity to protein contamination. For accurate A260/ A280 values, we recommend measuring absorbance in a slightly alkaline buffer (e.g., 10 mM Tris·Cl, pH 7.5~8.0). Make sure to zero the spectrophotometer with the appropriate buffer. Pure DNA has an A260/ A280 ratio of 1.7–1.9. Scanning the absorbance from 220–320 nm will show whether there are contaminants affecting absorbance at 260 nm. Absorbance scans should show a peak at 260 nm and an overall smooth shape.

RNA contamination

Depending on the DNA isolation method used, RNA will be copurified with genomic DNA. RNA may inhibit some downstream applications, but it will not inhibit PCR. Spectrophotometric measurements do not differentiate between DNA and RNA, so RNA contamination can lead to overestimation of DNA concentration.

TECHNICAL ADVICE

◈ General remarks on handling genomic DNA

DNA is a relatively stable molecule. However, introduction of nucleases to DNA solutions should be avoided as these enzymes will degrade DNA. Genomic DNA consists of very large DNA molecules, which are fragile and can break easily. To ensure the integrity of genomic DNA, excessive and rough pipetting and vortexing should be avoided. DNA is subject to acid hydrolysis when stored in water, and should therefore be stored in TE buffer or Buffer CE from iNtRON.

◈ Sample storage prior to isolation of genomic DNA

The quality of the starting material affects the quality and yield of the isolated DNA. The highest DNA yield and quality is achieved by purifying genomic DNA from freshly harvested tissues and cells. If samples cannot be processed immediately after harvesting, they should be stored under conditions that preserve DNA integrity. In general, genomic DNA yields will decrease if samples, particularly animal samples, are stored at either 2–8°C or –20°C without previous treatment. In addition, repeated freezing and thawing of frozen samples should be avoided as this will lead to genomic DNA of reduced size, and in clinical samples, to reduced yields of pathogen DNA (e.g., viral DNA).

Blood An anticoagulant should be added to blood samples that will be stored. For example, blood samples treated with heparin or EDTA can be stored at 2–8°C for a few days, or at –20°C or –80°C for a few weeks. Alternatively, blood samples can be treated with ACD Solution B (0.48% citric acid, 1.32% sodium citrate, 1.47% glucose; use 1 ml per 6 ml blood) and stored for at least 5 days at 2–8°C or 1 month at –20°C. For long-term storage, blood nuclei can be prepared and stored at –20°C.

Other clinical samples Most biological fluids (e.g., plasma, serum, and urine) and stool samples can be stored at 2–8°C for several hours. Freezing at –20°C or –80°C is recommended for long-term storage. Swabs can be stored dry at room temperature.

Animal tissue Freshly harvested tissue can be immediately frozen and stored at –20°C, –80°C, or in liquid nitrogen. Lysed tissue samples can be stored in a suitable lysis buffer for several months at ambient temperature. Animal and human tissues can also be fixed for storage. We recommend using fixatives such as alcohol and formalin; however, long-term storage of tissues in formalin will result in chemical modification of the DNA. Fixatives that cause cross-linking, such as osmic acid, are not recommended if DNA will be isolated from the tissue. It is also possible to isolate DNA from paraffin-embedded tissue.

Animal and bacterial cell cultures Centrifuge harvested cell cultures, remove the supernatant, and then store the cells at –20°C or –80°C. Alternatively, animal cell nuclei can be prepared and stored at –20°C.

APPLICATIONS

- Pathogen detection research
- Gram negative bacterial research
- SNP / Allele Research
- Very Sensitive Detection Assay : PCR, real time PCR
- DNA hybridization : Southern blotting, microarray
- Cancer research
- Paternity Research

PRODUCT WARRANTY AND SATISFACTION GUARANTEE

All products undergo extensive quality control test and are warranted to perform as described when used correctly. Immediately any problems should be reported. Satisfaction guarantee is conditional upon the customer providing full details of the problem to iNtRON within 60 days and returning the product to iNtRON for examination.

PRODUCT USE LIMITATIONS

The i-genomic Clinic DNA Extraction Mini Kit is intended for research use only. Prior to using it for other purposes, the user must validate the system in compliance with the applicable law, directives, and regulations.

i-genomic Clinic DNA Extraction Mini Kit is developed, designed, and sold for research purpose only. They are not to be used for human or animal diagnosis of diseases. Do not use internally or externally in humans or animals. Be careful in the handling of the products.

TECHNICAL ASSISTANCE

At iNtRON we pride ourselves on the quality and availability of our technical support. Our Technical Service Department is staffed by experienced scientists with extensive practical and theoretical expertise in molecular biology and the use of iNtRON products. If you have any questions or experience any difficulties regarding the i-genomic Clinic DNA Extraction Mini Kit or iNtRON products in general, please do not hesitate to contact us.

iNtRON customers are a major source of information regarding advanced or specialized uses of our products. This information is helpful to other scientists as well as to the researchers at iNtRON. We therefore encourage you to contact us if you have any suggestions about product performance or new applications and techniques.

For technical assistance and more information please call iNtRON Technical Service Department or local distributors.

PROTOCOL LIST

Table 1. Protocols according to the sample groups (8 Protocols)

Sample	Protocol Type
Blood, Body Fluids	Type A Protocol
Tissues	Type B Protocol
Swab (blood, buccal, fingerprint)	Type C Protocol
Stain/hair (hair root, hair shaft)/ nail clipping	Type D Protocol
Dried blood spot (blood card punches)	Type E Protocol
Fixed tissues	Type F Protocol
Cell/buffy coat	Type G Protocol
Bacteria	Type H Protocol
Cigarette butts	Type I Protocol
Chewing gum	Type J Protocol

SAMPLE PREPARATION

◈ Amounts of starting material

Use the amounts of starting material indicated in Table 2.

Table 2. Amounts of starting material for i-genomic Total Kit procedures

Sample	Amount	Sample	Amount
Blood, plasma, serum	1 ~ 200 µl	Cigarette butt	1 cm^2
Buffy coat	~ 200 µl	Chewing gum	30 mg
Tissue	~ 25 mg*	Stain	> 0.5 cm^2
Cultured cells	~ 1 x 10^6 cells	Hair root	> 0.5 - 1 cm
Bacterial culture (Liquid Culture)	~ 3 OD (OD_{600})	Hair shaft	> 0.5 - 1 cm
Swabs	1 ea	Nail Clipping	> small 1 piece

* When isolating DNA from spleen, 10 mg samples should be used.

TROUBLESHOOTING GUIDE

Problem	Possible Cause	Recommendation
A260/A280 ratio for purified nucleic acids is low	No ethanol added to the lysate before loading onto the column	Repeat the purification procedure with a new sample
	Buffers CWA and CWB used in the wrong order	Ensure that Buffers CWA and CWB are used in the correct order in the protocol. Repeat the purification procedure with a new Sample
White precipitate in Buffer CL or Buffer BL	White precipitate may form after storage at low temperature or prolonged storage	Any precipitate in Buffer CCL or Buffer CBL must be dissolved by incubation of the buffer at 56°C. The precipitate has no effect on function. Dissolving the precipitate at high temperature will not compromise yield or quality of the purified nucleic acid
General handling	Clogged membrane	Blood samples: Concentration of leukocytes in samples was greater than 5 x 10^6/200 µl. Dilute the sample with PBS and repeat the purification. Completely perform the Disruption & Homogenization step. And Increase the incubation time at 56°C in lysis step.
	Lysate not completely passed through the membrane	Using spin protocol: Centrifuge for 1 min at full speed or until all the lysate has passed through the membrane.
Low flow rate in column	Clogged Spin Column by particulate material	Completely perform the Disruption & Homogenization step. Or increase the incubation time at 56°C in Lysis step.
	High viscosity of Lysate Reagents correctly	Reduce the amounts of starting material. Or increase the incubation time at 56°C in Lysis step.
	Problem in centrifugation	Check your centrifuge, and then speed up or increase the centrifugation time.

TROUBLESHOOTING GUIDE

Problem	Possible Cause	Recommendation
Colored residues remain on the spin column after washing	Inefficient cell lysis due to insufficient mixing of the sample with Buffer CBL	Repeat the DNA purification procedure with a new sample. Be sure to mix the sample and Buffer CBL immediately and thoroughly by pulse-vortexing.
	Inefficient cell lysis due to decreased protease activity	Repeat the DNA purification procedure with a new sample and with freshly prepared Proteinase K stock solution. Be sure to store the stock solution at 2–8°C immediately after use. Ensure that Proteinase K is not added directly to Buffer CBL
	No ethanol added to the lysate before loading onto the column	Repeat the purification procedure with a new sample.
Little or no DNA in the eluate	Low concentration of cells or viruses in the sample	Concentrate a larger volume of a new cell- free sample to 200 µl using a Centricon®-100 (Amicon, USA). Repeat the DNA purification procedure, adding 5–10 µg of carrier to each lysate if the sample has a low DNA content. If whole blood was used, prepare buffy coat
	Inefficient cell lysis due to insufficient mixing with Buffer CBL	Repeat the DNA purification procedure with a new sample. Be sure to mix the sample and Buffer CBL immediately and thoroughly by pulse-vortexing.
	Inefficient cell lysis or protein degradation in Buffer CCL or Buffer CBL due to insufficient	Repeat the procedure with a new sample. Ensure that the tissue sample is cut into small pieces and extend the incubation time. incubation time Ensure that no residual particulates are Visible
	Low-percentage ethanol used instead of 100%.	Repeat the purification procedure with a new sample. Do not use denatured alcohol, which contains other substances such as methanol or methylethylketone
	pH of water incorrect (acidic)	Low pH may reduce DNA yield. Ensure that the pH of the water is at least 7.0 or use Buffer CE for elution.

◈ Preparation of buffy coat

Buffy coat is a leukocyte-enriched fraction of whole blood. Preparing a buffy-coat fraction from whole blood is simple and yields approximately 5–10 times more DNA than an equivalent volume of whole blood. Prepare buffy coat by centrifuging whole blood at 2500 x g for 10 minutes at room temperature. After centrifugation, 3 different fractions are distinguishable: the upper clear layer is plasma; the intermediate layer is buffy coat, containing concentrated leukocytes; and the bottom layer contains concentrated erythrocytes.

◈ Copurification of RNA

i-genomic Total Kit spin columns are able to copurify DNA and RNA when both are present in the sample (see Table 3). RNA may inhibit some downstream enzymatic reactions but will not inhibit PCR. If RNA contained genomic DNA is required, the treatment of RNase A should be bypassed to the sample.

Table 3. Composition of DNA and RNA from whole nucleic acid each of samples

Sample	DNA composition	RNA composition
Blood, plasma, serum, Buffy coat	95~100 %	0~5%
Tissues (Liver, Heart / Others)	15~20% / 40~50%	80~85%/50~60%
Cultured cells, Bacterial culture	30~50%	50~80%

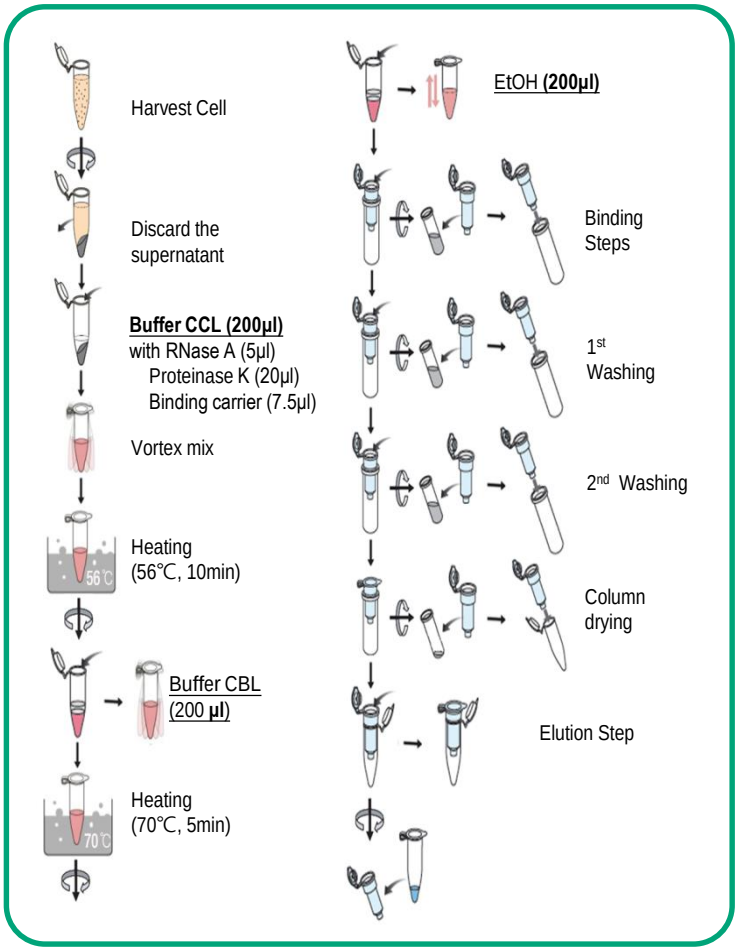
COLUMN INFORMATION

- The i-genomic Clinic DNA Extraction Mini Kit Spin Column

Column membrane[1]	Silica-based membrane
Spin Column[1]	Individually inserted in a 2.0 ml Collection Tube
Loading Volume	Maximum 800 µl
DNA Binding Capacity	Maximum 45 µg
Recovery	85 - 95% depending on the elution volume
Elution Volume	Generally, eluted with 30 – 200 µl of elution buffer

1. Do not store the Column packs under completely dried conditions. It may be affected to DNA binding capacity. The Spin Columns are stable for over 2 year under these conditions.

Quick Guide – Cell gDNA Extraction



9. Add 700 **µl** of Buffer CWB to the Spin Column, and centrifuge for 1 min at 13,000 rpm. Discard the flow-through and place the Column into a new 2.0 ml Collection Tube (additionally supplied). Then again centrifuge for additionally 1 min to dry the membrane. Discard the flow-through and Collection Tube altogether.
   Note : It is very important to dry the membrane of the Spin Column since residual ethanol may inhibit subsequent reactions. Following the centrifugation, remove carefully the Spin Column from the Collection Tube without contacting with the flow-through, since this will result in carryover of ethanol.
   Note : Ensure that 40 ml of absolute ethanol has been added to Buffer CWB.

10. Place the Spin Column into a new 1.5 ml tube (not supplied), and 30 - 100 **µl** of Buffer CE directly onto the membrane. Incubate for 1 min at room temperature and then centrifuge for 1 min at 13,000 rpm to elute.
   Note : Elution with 30 µl (instead of 50 µl) increases the final DNA concentration, but reduces overall DNA yield conventionally.
   Note : A new 1.5 ml tube can be used for the second elution step to prevent dilution of the first eluate. Alternatively, the tube can be reused for the second elution step to combine the eluates.

PROTOCOL J (Chewing gum)

1. Cut up to 30 mg of chewing gum into small pieces and transfer them to a 1.5 ml microcentrifuge tube (not provided).
2. Add 300 **µl** of Buffer CCL, 20 **µl** of Proteinase K Solution, 5 **µl** of RNase A and 7.5 **µl** Binding Carrier into sample tube and mix vortexing vigorously. Then Incubate the lysate at 56°C for 3 hr - overnight.
   Note : Be sure that Proteinase K solutions are always kept under freezer (below -10°C).
   Note : The Binding Carrier improves recovery of small amount of DNA.
   Note : Vortex the tube for 10 s every 10 min to improve lysis
3. Briefly centrifuge the 1.5 ml tube to remove drops from the inside of the rid.
4. Add 300 **µl** of Buffer CBL into the lysate, and mix well by gently inverting 5 - 6 times. After mixing, Then Incubate the lysate at 70°C for 10 min. Briefly centrifuge the 1.5 ml tube to remove drops from the inside of the rid.
5. Add 300 **µl** of absolute ethanol into the lysate, and mix well by gently inverting 5 - 6 times or by pipetting. DO NOT vortex. After mixing, Centrifuge at 13,000 rpm for 1 min.
   Note : This step is an equilibration step for binding genomic DNA to column membrane. It is important to assure proper mixing after adding the ethanol, until not showing 2-phase which is not mixed. Also, this step conduces to pass efficiently cell lysate through a column.
6. Carefully apply 800 **µl** of the supernatant from step 6 to the Spin Column (in a 2 ml Collection Tube) without wetting the rim, close the cap, and centrifuge at 13,000 rpm for 1 min. Discard the filtrate and place the Spin Column in a 2 ml Collection Tube (reuse).
   Note : Close each Spin Column in order to avoid aerosol formation during centrifugation. Do not transfer any solid materials.
7. Repeat step 7 by applying up to 100 **µl** of the remaining supernatant from step 6 to the Spin Column. Discard the filtrate and place the Spin Column in a new 2 ml Collection Tube (additionally supplied).
8. Add 700 **µl** of Buffer CWA to the Spin Column, and centrifuge for 1 min at 13,000 rpm. Discard the flow-through and reuse the Collection Tube.

PROTOCOL A (for Blood, body fluids)

1. Pipet 1-200 **µl** whole blood into a 1.5 ml microcentrifuge tube (not provided).
2. Add Buffer CCL to a final volume of 100 **µl**.
3. Add 20 **µl** of Proteinase K, 5 **µl** of RNase A Solution and 7.5 **µl** Binding Carrier into sample tube and gently mix.
   
   Note : It is possible to add Proteinase K to blood sample that have already been measured into 1.5 ml tube. It is important to assure proper mixing after adding the Proteinase K and RNase A solution.
   Note : The Binding Carrier improves recovery of small amount of DNA. Be sure that Proteinase K solutions are always kept under freezer (below -10°C).
4. Add 200 **µl** of Buffer CBL into upper sample tube and mix thoroughly.
   
   Note : Avoid any vigorous vortexing because doing so may induce genomic DNA breakage. In order to assure efficient lysis, it is important that the blood sample and Buffer CBL are mixed thoroughly to yield a lysis solution.
5. Incubate the lysate at 56°C for 10 min.
   Note : For complete lysis, mix 3 or 4 times during incubation by inverting tube. If it lysis perfectly, the red color of lysate becomes the dark green.
6. Briefly centrifuge the 1.5 ml tube to remove drops from the inside of the rid.
7. Add 300 **µl** of absolute ethanol into the lysate, and mix well by gently inverting 5 - 6 times or by pipetting. DO NOT vortex. After mixing, briefly centrifuge the 1.5 ml tube to remove drops from inside of the lid.
   Note : This step is an equilibration step for binding genomic DNA to column membrane. It is important to assure proper mixing after adding the ethanol, until not showing 2-phase which is not mixed. Also, this step conduces to pass efficiently cell lysate through a column.
8. Carefully apply the mixture from step 7 to the Spin Column (in a 2 ml Collection Tube) without wetting the rim, close the cap, and centrifuge at 13,000 rpm for 1 min. Discard the filtrate and place the Spin Column in a new 2 ml Collection Tube (additionally supplied).
   Note : Close each Spin Column in order to avoid aerosol formation during centrifugation. Do not transfer any solid materials.
9. Add 700 **µl** of Buffer CWA to the Spin Column, and centrifuge for 1 min at 13,000 rpm. Discard the flow-through and reuse the Collection Tube.

10. Add 700 **µl** of Buffer CWB to the Spin Column, and centrifuge for 1 min at 13,000 rpm. Discard the flow-through and place the Column into a new 2.0 ml Collection Tube (additionally supplied). Then again centrifuge for additionally 1 min to dry the membrane. Discard the flow-through and Collection Tube altogether.
    Note : It is very important to dry the membrane of the Spin Column since residual ethanol may inhibit subsequent reactions. Following the centrifugation, remove carefully the Spin Column from the Collection Tube without contacting with the flow-through, since this will result in carryover of ethanol.
    Note : Ensure that 40 ml of absolute ethanol has been added to Buffer CWB.

11. Place the Spin Column into a new 1.5 ml tube (not supplied), and 30 - 100 **µl** of Buffer CE directly onto the membrane. Incubate for 1 min at room temperature and then centrifuge for 1 min at 13,000 rpm to elute.
    Note : Elution with 30 µl (instead of 50 µl) increases the final DNA concentration, but reduces overall DNA yield conventionally.
    Note : A new 1.5 ml tube can be used for the second elution step to prevent dilution of the first eluate. Alternatively, the tube can be reused for the second elution step to combine the eluates.

11. Repeat step 10 by applying up to 100 **µl** of the remaining mixture from step 9 to the Spin Column. Discard the filtrate and place the Spin Column in a new 2 ml Collection Tube (additionally supplied).

12. Add 700 **µl** of Buffer CWA to the Spin Column, and centrifuge for 1 min at 13,000 rpm. Discard the flow-through and reuse the Collection Tube.

13. Add 700 **µl** of Buffer CWB to the Spin Column, and centrifuge for 1 min at 13,000 rpm. Discard the flow-through and place the Column into a new 2.0 ml Collection Tube (additionally supplied). Then again centrifuge for additionally 1 min to dry the membrane. Discard the flow-through and Collection Tube altogether.
    Note : It is very important to dry the membrane of the Spin Column since residual ethanol may inhibit subsequent reactions. Following the centrifugation, remove carefully the Spin Column from the Collection Tube without contacting with the flow-through, since this will result in carryover of ethanol.
    Note : Ensure that 40 ml of absolute ethanol has been added to Buffer CWB.

14. Place the Spin Column into a new 1.5 ml tube (not supplied) and 30 - 100 **µl** of Buffer CE directly onto the membrane. Incubate for 1 min at room temperature and then centrifuge for 1 min at 13,000 rpm to elute.
    Note : Elution with 30 µl (instead of 50 µl) increases the final DNA concentration, but reduces overall DNA yield conventionally.
    Note : A new 1.5 ml tube can be used for the second elution step to prevent dilution of the first eluate. Alternatively, the tube can be reused for the second elution step to combine the eluates.

PROTOCOL I (Cigarette butts)

1. Prepare sample.
   Note : Air-dry the cigarette butts for at least 2 hr after collection. After sample collection, samples can be kept at room temperature when processed immediately.
2. Place small pieces of cigarette butts into a 1.5 ml micro-centrifuge tube.
   Note : Cigarette butts are cut out a 1 cm^2 piece of outer paper from the end of the cigarette filter. Cut this piece into 6 smaller pieces. Transfer the pieces to a 1.5 ml microcentrifuge tube
3. Add 300 **µl** of Buffer CCL, 20 **µl** of Proteinase K Solution, 5 **µl** of RNase A and 7.5 **µl** Binding Carrier into sample tube and mix vortexing vigorously.
   Note : Be sure that Proteinase K solutions are always kept under freezer (below -10°C).
   Note : The Binding Carrier improves recovery of small amount of DNA.
4. Incubate the lysate at 56°C for 3 hr - overnight.
   Note : If using a heating block or water bath, vortex the tube for 10 s every hours to improve lysis.
5. Briefly centrifuge the 1.5 ml tube to remove drops from the inside of the rid.
6. Add 300 **µl** of Buffer CBL into the lysate, and mix well by gently inverting 5 - 6 times.
7. After mixing, incubate the lysate at 70°C for 10 min.
8. Briefly centrifuge the 1.5 ml tube to remove drops from the inside of the rid.
9. Add 300 **µl** of absolute ethanol into the lysate, and mix well by gently inverting 5 - 6 times or by pipetting. DO NOT vortex. After mixing, briefly centrifuge the 1.5 ml tube to remove drops from inside of the lid.
   Note : This step is an equilibration step for binding genomic DNA to column membrane. It is important to assure proper mixing after adding the ethanol, until not showing 2-phase which is not mixed. Also, this step conduces to pass efficiently cell lysate through a column.
10. Carefully apply 800 **µl** of the mixture from step 7 to the Spin Column (in a 2 ml Collection Tube) without wetting the rim, close the cap, and centrifuge at 13,000 rpm for 1 min. Discard the filtrate and place the Spin Column in a 2 ml Collection Tube (reuse).
    Note : Close each Spin Column in order to avoid aerosol formation during centrifugation. Do not transfer any filter paper.

PROTOCOL B (for Tissue)

1. Transfer a tissue sample of less than 25mg in weight to a 1.5ml microcentrifuge tube (not provided).
2. Add 200 **µl** of Buffer CCL, 20 **µl** of Proteinase K Solution, 5 **µl** of RNase A and 7.5 **µl** Binding Carrier into sample tube and mix vortexing vigorously. Then Incubate the lysate at 56°C for overnight.
   Note : For small amounts of tissue, lysis is complete in 4–6 hr, but best results are achieved after overnight lysis. Vortex the tube for 10 s every 1-2 hr to improve lysis.
3. Briefly centrifuge the 1.5 ml tube to remove drops from the inside of the rid.
4. Add 200 **µl** of Buffer CBL into the lysate, and mix well by gently inverting 5 - 6 times. After mixing, incubate the lysate at 65°C for 10 min.
5. Briefly centrifuge the 1.5 ml tube to remove drops from the inside of the rid.
6. Add 200 **µl** of absolute ethanol into the lysate and mix well by gently inverting 5 - 6 times or by pipetting. DO NOT vortex. After mixing, centrifuge at 13,000 rpm for 1 min.
   Note : This step is an equilibration step for binding genomic DNA to column membrane. It is important to assure proper mixing after adding the ethanol, until not showing 2-phase which is not mixed. Also, this step conduces to pass efficiently cell lysate through a column.
7. Carefully apply 600 **µl** of the supernatant from step 6 to the Spin Column (in a 2 ml Collection Tube) without wetting the rim, close the cap, and centrifuge at 13,000 rpm for 1 min. Discard the filtrate and place the Spin Column in a 2 ml Collection Tube (Reuse).
   Note : Close each Spin Column in order to avoid aerosol formation during centrifugation. Do not transfer any solid materials.
8. Repeat by applying up to 100 **µl** of the remaining supernatant from step 6 to the Spin Column.
9. Place the Spin Column into a new 2.0 ml Collection Tube (additionally supplied), add 700 **µl** of Buffer CWA to the Spin Column, and centrifuge for 1 min at 13,000 rpm. Discard the flow-through and reuse the Collection Tube.

10. Add 700 **µl** of Buffer CWB to the Spin Column and centrifuge for 1 min at 13,000 rpm. Discard the flow-through and place the Column into a new 2.0 ml Collection Tube (additionally supplied). Then again centrifuge for additional 1 min to dry the membrane. Discard the flow-through and Collection Tube altogether.

Note : It is very important to dry the membrane of the Spin Column since residual ethanol may inhibit subsequent reactions. Following the centrifugation, remove carefully the Spin Column from the Collection Tube without contacting with the flow-through, since this will result in carryover of ethanol.

Note : Ensure that 40 ml of absolute ethanol has been added to Buffer CWB.

11. Place the Spin Column into a new 1.5 ml tube (not supplied), and 30 - 100 **µl** of Buffer CE directly onto the membrane. Incubate for 1 min at room temperature and then centrifuge for 1 min at 13,000 rpm to elute.

Note : Elution with 30 µl (instead of 50 µl) increases the final DNA concentration, but reduces overall DNA yield conventionally.

Note : A new 1.5 ml tube can be used for the second elution step to prevent dilution of the first eluate. Alternatively, the tube can be reused for the second elution step to combine the eluates.

PROTOCOL H (Gram Negative Bacteria)

1. Prepare Gram negative bacteria sample.

Note : Streak or spread cell on solid media plate (ex. LB, SOB etc.) . Incubate for 14 ~ 16hr at 37℃. Pick up the single colony from media plate. Inoculate single colony to 5 ml liquid culture media (ex. LB, SOB etc), then incubate for overnight at 37℃ until OD600 value of 0.8 ~ 1.0 on a spectrophotometer. OD600 values depend on the length of the light path and therefore differ between spectrophotometers.

2. Transfer 1 ~ 2 ml cultured bacteria cell into 2 ml tube.

Note : If an excess of starting amount is applied more than the recommended optimal amount of starting material, it will result in inefficient lysis, resulting in low yield and purity.

3. Pellet bacteria by centrifugation for 1 min at 13,000 rpm, and discard supernatant. Resuspend compeletely the cell pellet with remnant supernatant by tapping or vigorously vortexing.

Note : It is essential that the pellet and remnant supernatant are mixed thoroughly yield a homogeneous solution.

4. Add 200 **µl** Buffer CCL, 20 **µl** Proteinase K, 5 **µl** RNase A Solution and 7.5 **µl** of binding carrier into sample tube and mix vortexing vigorously.

Note : Be sure that Proteinase K and RNase A solutions are always kept under freezer (below -10℃).

5. Incubate lysate at 56**℃** (preheated heat block or water bath) for 10 ~ 30 min.

Note : To help lysis sample, mix the tube by inverting every 2 min during the incubation. In case of gram negative bacteria sample, it is enough to lysis for 10 ~ 20 min, respectively.

6. After lysis completely, add 200 **µl** of Buffer CBL into upper sample tube and mix thoroughly. Then incubate the mixture at 70**℃** for 5min.

Note : Avoid any vigorous vortexing because doing so many induce genomic DNA breakage. In order to assure efficient lysis, it is important that the lysate sample and Buffer BL are mixed thoroughly.

7. Centrifuge the sample tube at 13,000 rpm for 5 min to remove un-lysed particles. Then carefully transfer 350 ~ 400 **µl** of the supernatant into a new 1.5 ml tube (not provided).

Note : If insoluble clumps remains in homogenated mixture, it will be occurred spin column clogging, sometimes. This step helps sample mixing with buffer during binding step by and large. Also It prevents column clogging from insoluble clumps.

8. Follow the Protocol I (for Cell, Buffy coat) from Step 8.

12. Add 700 **µl** of Buffer CWB to the Spin Column, and centrifuge for 1 min at 13,000 rpm. Discard the flow-through and place the Column into a new 2.0 ml Collection Tube (additionally supplied). Then again centrifuge for additionally 1 min to dry the membrane. Discard the flow-through and Collection Tube altogether.
    Note : It is very important to dry the membrane of the Spin Column since residual ethanol may inhibit subsequent reactions. Following the centrifugation, remove carefully the Spin Column from the Collection Tube without contacting with the flow-through, since this will result in carryover of ethanol.
    Note : Ensure that 40 ml of absolute ethanol has been added to Buffer CWB.

13. Place the Spin Column into a new 1.5 ml tube (not supplied), and 30 - 100 **µl** of Buffer CE directly onto the membrane. Incubate for 1 min at room temperature and then centrifuge for 1 min at 13,000 rpm to elute.
    Note : Elution with 30 µl (instead of 50 µl) increases the final DNA concentration, but reduces overall DNA yield conventionally.
    Note : A new 1.5 ml tube can be used for the second elution step to prevent dilution of the first eluate. Alternatively, the tube can be reused for the second elution step to combine the eluates.

PROTOCOL C (Swab (blood, buccal, fingerprint))

1. Prepare sample.
    Note : To collect a sample, scrape the swab firmly against the surface of each sample more than 6 times. Air-dry the swab for at least 2 hr after collection. After sample collection, samples can be kept at room temperature when processed immediately. If storage is necessary, freeze swab sample at - 20 °C.

2. Place single swab into a 1.5 ml micro-centrifuge tube.
    Note : Cotton or DACRON swabs are cut from the stick by scissors.

3. Add 400 **µl** of Buffer CCL, 20 **µl** of Proteinase K Solution, 5 **µl** of RNase A and 7.5 **µl** Binding Carrier into sample tube and mix vortexing vigorously. Then Incubate the lysate at 56°C at least 1 hr.
    Note : Be sure that Proteinase K and RNase A solutions are always kept under freezer (below -10°C).
    Note : The Binding Carrier improves precipitation of small amount of DNA was optimize DNA recovery.

4. Briefly centrifuge the 1.5 ml tube to remove drops from the inside of the rid.

5. Add 400 **µl** of Buffer CBL into the lysate, and mix well by gently inverting 5 - 6 times. After mixing, Then Incubate the lysate at 70°C for 10 min.

6. Briefly centrifuge the 1.5 ml tube to remove drops from the inside of the rid.

7. Add 400 **µl** of absolute ethanol into the lysate, and mix well by gently inverting 5 - 6 times or by pipetting. DO NOT vortex. After mixing, briefly centrifuge the 1.5 ml tube to remove drops from inside of the lid.
    Note : This step is an equilibration step for binding genomic DNA to column membrane. It is important to assure proper mixing after adding the ethanol, until not showing 2-phase which is not mixed. Also, this step conduces to pass efficiently cell lysate through a column.

8. Carefully apply 800 **µl** of the mixture from step 7 to the Spin Column (in a 2 ml Collection Tube) without wetting the rim, close the cap, and centrifuge at 13,000 rpm for 1 min. Discard the filtrate and place the Spin Column in a 2 ml Collection Tube (reuse).
    Note : Close each Spin Column in order to avoid aerosol formation during centrifugation. Do not transfer any solid materials.

9. Repeat step 8 by applying up to 500 - 600 **µl** of the remaining mixture from step 7 to the Spin Column. Discard the filtrate and place the Spin Column in a new 2 ml Collection Tube (additionally supplied).

10. Add 700 **µl** of Buffer CWA to the Spin Column, and centrifuge for 1 min at 13,000 rpm. Discard the flow-through and reuse the Collection Tube.

11. Add 700 **µl** of Buffer CWB to the Spin Column, and centrifuge for 1 min at 13,000 rpm. Discard the flow-through and place the Column into a new 2.0 ml Collection Tube (additionally supplied), Then again centrifuge for additionally 1 min to dry the membrane. Discard the flow-through and Collection Tube altogether.

Note : It is very important to dry the membrane of the Spin Column since residual ethanol may inhibit subsequent reactions. Following the centrifugation, remove carefully the Spin Column from the Collection Tube without contacting with the flow-through, since this will result in carryover of ethanol.
Note : Ensure that 40 ml of absolute ethanol has been added to Buffer CWB.

12. Place the Spin Column into a new 1.5 ml tube (not supplied), and 30 - 100 **µl** of Buffer CE directly onto the membrane. Incubate for 1 min at room temperature and then centrifuge for 1 min at 13,000 rpm to elute.
Note : Elution with 30 µl (instead of 50 µl) increases the final DNA concentration, but reduces overall DNA yield conventionally.
Note : A new 1.5 ml tube can be used for the second elution step to prevent dilution of the first eluate. Alternatively, the tube can be reused for the second elution step to combine the eluates.

4. Add 200 **µl** Buffer CCL, 20 **µl** Proteinase K and 5 **µl** RNase A Solution into sample tube and mix by vortexing vigorously.

Note : Be sure that Proteinase K and RNase A solutions are always kept under freezer (below -10℃). In case of transcriptionally active cultured cell, contain large amount of RNA which will be copurified with genomic DNA. RNA may inhibit downstream enzymatic reaction, but will not affect PCR.

5. Incubate the lysate at 56**℃** (preheated heat block or water bath) for 10 ~ 30 min.
Note : To help lysis tissue sample, mix the tube by inverting every 2 min during the incubation. Lysis time varies depending on the type of sample. In case of cultured cell, it is enough to lysis completely for 10 ~ 15 min, respectively.

6. After lysis completely, add 200 **µl** of Buffer CBL into upper sample tube and mix thoroughly. Then incubate the mixture at 70**℃** for 5min.

Note : Avoid any vigorous vortexing because doing so many induce genomic DNA breakage. In order to assure efficient lysis, it is important that the lysate sample and Buffer CBL are mixed thoroughly.

7. Briefly centrifuge the 1.5 ml tube to remove drops from the inside of the rid.

8. Add 200 **µl** of absolute ethanol into the lysate, and mix well by gently inverting 5 - 6 times or by pipetting. DO NOT vortex. After mixing, Centrifuge at 13,000 rpm for 1 min.
Note : This step is an equilibration step for binding genomic DNA to column membrane. It is important to assure proper mixing after adding the ethanol, until not showing 2-phase which is not mixed. Also, this step conduces to pass efficiently cell lysate through a column.

9. Carefully apply 600 **µl** of the supernatant from step 6 to the Spin Column (in a 2 ml Collection Tube) without wetting the rim, close the cap, and centrifuge at 13,000 rpm for 1 min. Discard the filtrate and place the Spin Column in a 2 ml Collection Tube (reuse).
Note : Close each Spin Column in order to avoid aerosol formation during centrifugation. Do not transfer any solid materials.

10. Repeat by applying up to 100 **µl** of the remaining supernatant from step 6 to the Spin Column.

11. Place the Spin Column into a new 2.0 ml Collection Tube (additionally supplied), add 700 **µl** of Buffer CWA to the Spin Column, and centrifuge for 1 min at 13,000 rpm. Discard the flow-through and reuse the Collection Tube.

PROTOCOL G (Cell, Buffy coat)

1. Prepare the sample according to 1a, 1b or 1c.

1a. Cells grown in suspension ; Transfer the culture fluid into 15 ml or 50 ml of centrifuge tube and pellet the culture by centrifugation for 5 min at 3,000 rpm. Remove the supernatant completely and wash the pellet with PBS or fresh media. Then resuspend the washed cell pellet in appropriate volume of PBS or fresh media.

1b. Cells grown in monolayer ; Cells grown in monolayer can be detached from culture flask (or plate) by either 1) Trypsinization or 2) Using a cell scraper.

1) To Trypsinize cells : Remove the medium and wash the cells with preheated (at 37℃) PBS. Then aspirate the PBS and add trypsin solution. After cells have become detached from culture flask (or dish), collect and wash the cells with PBS, then resuspend the washed cell pellet in appropriate volume of PBS or fresh media.

2) Using a cell scrape, detach cells from culture flask or dish. Collect and wash the cells with PBS, then resuspend the washed cell pellet in appropriate volume of PBS or fresh media.

1c. Buffy coat preparation ; Buffy coat is the fraction of a centrifugated blood sample that contain most of the white blood cells. To get the buffy coat, it is better to use after obtain whole blood immediately. Prepare buffy coat by centrifuging whole blood at 3,000 rpm for 10 minutes at room temperature. After centrifugation, one can distinguish a layer of clear fluid (the plasma), a layer of red fluid containing most of the red blood cells, and a thin layer in between, the buffy coat, with most of the white blood cells and platelets. The buffy coat is usually whitish in color but sometimes green. The buffy coat is used to extract DNA from the blood of mammals. The yields are approximately 6 ~ 10 times more DNA than an equivalent volume of whole blood. After then, measure 200 µl of buffy coat, and then transfer into 1.5 ml tube using a micro pipette.

2. Determinate the cell number using cell counter (eg. hemocytometer) and transfer the appropriated number of cells (1 ~ 3 x 106 cells) to a new 1.5 ml microcentrifuge tube. (in case of buffy coat, bypass the step 2, 3)

3. Pellet the cell by centrifugation for 1 min at 13,000 rpm and discard the supernatant. Then resuspend completely the cell pellet with remnant supernatant by tapping or vigorously vortexing.
Note : In order to ensure efficient lysis, it is essential that the cell pellet and remnant supernatant are mixed thoroughly to yield a homogeneous solution.

PROTOCOL D (Stain/hair (hair root, hair shaft)/ nail clipping)

1. Prepare sample
   1) Stains (stained with blood, saliva or semen) : Cut out up to 0.5 cm^2 of stained material and then cut it into smaller pieces. Transfer the pieces to a 2 ml microcentrifuge tube (not provided).
   2) Hair root : Cut off a 0.5 - 1 cm piece starting from the hair bulb and transfer it to the 1.5 ml microcentrifuge tube not provided).
   3) Hair shaft : Cut the hair shaft into 0.5 - 1 cm pieces and transfer them to 1.5 ml microcentrifuge tube (not provided). Close the lid and mix by pulse-vortexing for 10 s.
   4) Nail Clipping : Transfer the nail clippings to a 1.5 ml microcentrifuge tube (not provided).

2. Add 300 **µl** of Buffer CCL, 20 **µl** of Proteinase K Solution, 5 **µl** of RNase A, 20 **µl** of 1M DTT (not provided) and 7.5 **µl** Binding Carrier into sample tube and mix vortexing vigorously. Then Incubate the lysate at 56**°C** at least 1 hr.
Note : In general, hairs are lysed in 1 hr. If necessary, increase the incubation time to ensure complete lysis. If using a heating block or water bath, vortex the tube for 10 s every 10 min to improve lysis.

Note : For larger samples of nail clippings, we recommend overnight incubation at 56°C. Any material that is not lysed during this incubation step or the incubation in step 4 will be pelleted during centrifugation in step 5.

3. Briefly centrifuge the 1.5 ml tube to remove drops from the inside of the rid.

4. Add 300 **µl** of Buffer CBL into the lysate, and mix well by gently inverting 5 - 6 times. After mixing, incubate the lysate at 70**°C** for 10 min.

5. Briefly centrifuge the 1.5 ml tube to remove drops from the inside of the rid.

6. Add 300 **µl** of absolute ethanol into the lysate and mix well by gently inverting 5 - 6 times or by pipetting. DO NOT vortex. After mixing, briefly centrifuge the 1.5 ml tube to remove drops from inside of the lid.

7. Carefully apply 800 **µl** of the mixture from step 7 to the Spin Column (in a 2 ml Collection Tube) without wetting the rim, close the cap, and centrifuge at 13,000 rpm for 1 min. Discard the filtrate and place the Spin Column in a 2 ml Collection Tube (reuse).
Note : Close each Spin Column in order to avoid aerosol formation during centrifugation. Do not transfer any solid materials.

8. Repeat step 7 by applying up to 100 **µl** of the remaining mixture from step 6 to the Spin Column. Discard the filtrate and place the Spin Column in a new 2 ml Collection Tube (additionally supplied).

9. Add 700 **µl** of Buffer CWA to the Spin Column, and centrifuge for 1 min at 13,000 rpm. Discard the flow-through and reuse the Collection Tube.

10. Add 700 **µl** of Buffer CWB to the Spin Column, and centrifuge for 1 min at 13,000 rpm. Discard the flow-through and place the Column into a new 2.0 ml Collection Tube (additionally supplied), Then again centrifuge for additionally 1 min to dry the membrane. Discard the flow-through and Collection Tube altogether.

Note : It is very important to dry the membrane of the Spin Column since residual ethanol may inhibit subsequent reactions. Following the centrifugation, remove carefully the Spin Column from the Collection Tube without contacting with the flow-through, since this will result in carryover of ethanol.

Note : Ensure that 40 ml of absolute ethanol has been added to Buffer CWB.

11. Place the Spin Column into a new 1.5 ml tube (not supplied), and 30 - 100 **µl** of Buffer CE directly onto the membrane. Incubate for 1 min at room temperature and then centrifuge for 1 min at 13,000 rpm to elute.

Note : Elution with 30 µl (instead of 50 µl) increases the final DNA concentration, but reduces overall DNA yield conventionally.

Note : A new 1.5 ml tube can be used for the second elution step to prevent dilution of the first eluate. Alternatively, the tube can be reused for the second elution step to combine the eluates.

21. Add 700 **µl** of Buffer CWB to the Spin Column, and centrifuge for 1 min at 13,000 rpm. Discard the flow-through and place the Column into a new 2.0 ml Collection Tube (additionally supplied), Then again centrifuge for additionally 1 min to dry the membrane. Discard the flow-through and Collection Tube altogether.

Note : It is very important to dry the membrane of the Spin Column since residual ethanol may inhibit subsequent reactions. Following the centrifugation, remove carefully the Spin Column from the Collection Tube without contacting with the flow-through, since this will result in carryover of ethanol.

Note : Ensure that 40 ml of absolute ethanol has been added to Buffer CWB.

22. Place the Spin Column into a new 1.5 ml tube (not supplied), and 30 - 100 **µl** of Buffer CE directly onto the membrane. Incubate for 1 min at room temperature and then centrifuge for 1 min at 13,000 rpm to elute.

Note : Elution with 30 µl (instead of 50 µl) increases the final DNA concentration, but reduces overall DNA yield conventionally.

Note : A new 1.5 ml tube can be used for the second elution step to prevent dilution of the first eluate. Alternatively, the tube can be reused for the second elution step to combine the eluates.

12. Add 150 **µl** Buffer CCL, 20 **µl** of Proteinase K, 5 **µl** of RNase A Solution and 7.5 **µl** Binding Carrier into sample tube and mix by vortexing vigorously. Then Incubate the lysate at 56°C for overnight.
    Note : Be sure that Proteinase K and RNase A solutions are always kept under freezer (below -10℃). In case of transcriptionally active tissues, such as liver and kidney, contain large amount of RNA which will be copurified with genomic DNA. RNA may inhibit downstream enzymatic reaction, but will not affect PCR
13. Briefly centrifuge the 1.5 ml tube to remove drops from the inside of the rid.
14. Add 200 **µl** of Buffer CBL into the lysate, and mix well by gently inverting 5 - 6 times. After mixing, Then Incubate the lysate at 70°C for 10 min.
15. Briefly centrifuge the 1.5 ml tube to remove drops from the inside of the rid.
16. Add 200 **µl** of absolute ethanol into the lysate and mix well by gently inverting 5 - 6 times or by pipetting. DO NOT vortex. After mixing, centrifuge at 13,000 rpm for 1 min.
    Note : This step is an equilibration step for binding genomic DNA to column membrane. It is important to assure proper mixing after adding the ethanol, until not showing 2-phase which is not mixed. Also, this step conduces to pass efficiently cell lysate through a column.
17. Carefully apply 600 **µl** of the supernatant from step 6 to the Spin Column (in a 2 ml Collection Tube) without wetting the rim, close the cap, and centrifuge at 13,000 rpm for 1 min. Discard the filtrate and place the Spin Column in a 2 ml Collection Tube (reuse).
    Note : Close each Spin Column in order to avoid aerosol formation during centrifugation. Do not transfer any solid materials.
18. Repeat by applying up to 100 **µl** of the remaining supernatant from step 6 to the Spin Column.
19. Place the Spin Column into a new 2.0 ml Collection Tube (additionally supplied), add 700 **µl** of Buffer CWA to the Spin Column and centrifuge for 1 min at 13,000 rpm. Discard the flow-through and reuse the Collection Tube.

PROTOCOL E (Dried blood spot (blood card punches))

1. Cut 3 mm (1/8 inch) diameter punches from a dried blood spot with a single-hole paper punch. Place up to 3 blood card punches into a 1.5 ml microcentrifuge tube (not provided).
2. Add 200 **µl** of Buffer CCL, 20 **µl** of Proteinase K Solution, 5 **µl** of RNase A and 7.5 **µl** Binding Carrier into sample tube and mix vortexing vigorously. Then Incubate the lysate at 56°C at least 1 hr.
   Note : If using a heating block or water bath, vortex the tube for 10 s every 10 min to improve lysis.
3. Briefly centrifuge the 1.5 ml tube to remove drops from the inside of the rid.
4. Add 200 **µl** of Buffer CBL into the lysate and mix well by gently inverting 5 - 6 times. After mixing, incubate the lysate at 70°C for 10 min.
5. Briefly centrifuge the 1.5 ml tube to remove drops from the inside of the rid.
6. Add 200 **µl** of absolute ethanol into the lysate, and mix well by gently inverting 5 - 6 times or by pipetting. DO NOT vortex. After mixing, briefly centrifuge the 1.5 ml tube to remove drops from inside of the lid.
7. Carefully apply 600 **µl** of the mixture from step 6 to the Spin Column (in a 2 ml Collection Tube) without wetting the rim, close the cap and centrifuge at 13,000 rpm for 1 min. Discard the filtrate and place the Spin Column in a new 2 ml Collection Tube (additionally supplied).
   Note : Close each Spin Column in order to avoid aerosol formation during centrifugation. Do not transfer any solid materials.
8. Add 700 **µl** of Buffer CWA to the Spin Column, and centrifuge for 1 min at 13,000 rpm. Discard the flow-through and reuse the Collection Tube.
9. Add 700 **µl** of Buffer CWB to the Spin Column, and centrifuge for 1 min at 13,000 rpm. Discard the flow-through and place the Column into a new 2.0 ml Collection Tube (additionally supplied), Then again centrifuge for additionally 1 min to dry the membrane. Discard the flow-through and Collection Tube altogether.

Note : It is very important to dry the membrane of the Spin Column since residual ethanol may inhibit subsequent reactions. Following the centrifugation, remove carefully the Spin Column from the Collection Tube without contacting with the flow-through, since this will result in carryover of ethanol.
Note : Ensure that 40 ml of absolute ethanol has been added to Buffer CWB.

10. Place the Spin Column into a new 1.5 ml tube (not supplied), and 30 - 100 **µl** of Buffer CE directly onto the membrane. Incubate for 1 min at room temperature and then centrifuge for 1 min at 13,000 rpm to elute.
    Note : Elution with 30 µl (instead of 50 µl) increases the final DNA concentration, but reduces overall DNA yield conventionally.
    Note : A new 1.5 ml tube can be used for the second elution step to prevent dilution of the first eluate. Alternatively, the tube can be reused for the second elution step to combine the eluates.

PROTOCOL F (Fixed tissues)

- Paraffin embedded block : follow the protocol from step 1
- Formalin fixed tissue : follow the protocol from step 6

1. Slice of the paraffin block into thin pieces.
2. Place a small section (not more than 25 mg) of Paraffin fixed tissue in a 2.0 ml tube.
   Note : Ensure that the correct amount of starting material is used. If the genomic DNA is prepared from spleen or thymus tissue, no more than 10 mg should be used. To maximize the purification yield, remove the paraffin only part as possible.
3. Add 1.2 ml xylene and mix by vortexing vigorously. Then centrifuge at full speed for 5 min at room temperature.
   Note : This step describes the removal of paraffin by extraction with xylene.
4. Remove supernatant by pipetting. Do not remove any of the pellet.
5. Repeat the step 3 ~ 4 once.
6. Add 1.2 ml absolute ethanol and mix by vortexing vigorously. Then centrifuge at full speed for 5 min at room temperature.
7. Remove supernatant by pipetting. Do not remove any of the pellet.
8. Repeat the step 6 ~ 7 once.
9. Incubate the open tube at 65°C for 10–15 min until the ethanol has evaporated completely.
   Note : It is the most important key point of DNA extraction from paraffin embedded tissue to remove the remnant ethanol.
10. Transfer the dried tissue sample into a new 1.5ml tube.
11. Crash the tissue 10 ~ 20 times using pestle without buffer adding. After preliminary crashing, add 50 **µl** of Buffer CCL to the sample tube. Keep the sample submerged in Buffer CCL, and disrupt carefully until the sample is homogenized completely.
    Note : Disruption and homogenization time depends on the tissue samples. We recommend to be disrupted completely until no tissue clumps are not visible. Clumps of tissue sample will not lyse properly and will therefore result in a lower yield of DNA.