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Speed

Takes only 20 ~ 30 minutes to extract genomic DNA.

Smart

High quality and quantity of DNA recovery

Steady

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

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.

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Instruction manual

G-spin™ Total DNA Extraction Kit

The Instruction Manual for Genomic DNA Extraction from Cultivated animal cells, tissues, Gram negative bacteria and Blood using silica membrane.

REF	17045	Σ 50
REF	17046	Σ 200
RUO		
RT		

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INDEX

DESCRIPTION

- G-spin™ Total DNA Extraction Mini Kit provide fast and easy methods for purification of total DNA from cultured animal cell, animal tissue, rodent tail, fixed tissue, animal hair, gram negative bacteria, and blood samples for reliable PCR and Southern blotting. Furthermore, we have tested G-spin™ Total DNA Extraction Mini Kit to get more practical data with a lot numbers of biological samples.
- The simple G-spin™ Total DNA Extraction procedures, which are ideal for simultaneous processing of multiple samples, yield pure DNA ready for direct amplification in just 20 ~ 30 minutes. The G-spin™ Total DNA Extraction Mini Kit procedure is suitable for use with fresh or frozen whole blood and blood which has been treated with citrate, heparin, or EDTA. Pre-separation of leukocytes is not necessary.
- Purification requires no phenol/chloroform extraction or alcohol precipitation, and involves very little handling. DNA is eluted in Buffer CE, TE (10:1), 10mM Tris (pH 7.5 ~ 8) or water, ready for direct addition to PCR or other enzymatic reactions. Alternatively, it can be safely stored at –20 ℃ for later use. The purified DNA is free of protein, nucleases, and other contaminants or inhibitors. DNA purified using G-spin™ Total DNA Extraction Mini Kit is up to 50 kb in size, with fragments of approximately 20–30 kb predominating. DNA of this length denatures completely during thermal cycling and can be amplified with high efficiency.
- G-spin™ Total DNA Extraction Mini Kit provides various protocols. You can also extract genomic DNA from various your biological samples by selecting an appropriate protocol from Protocol list (see Table 1). If you need some more information in selecting a protocol, please do not hesitate to contact our Technical Assistance Team.

CHARACTERISTICS

- **Speed** : Takes only 20 ~ 30 minutes to extract genomic DNA.
- **Smart** : High quality and quantity of DNA recovery
- **Steady** : Complete removal of inhibitors and contaminants for accurate down stream applications. And the freeze-dried formulated enzyme has been improved DNA extraction stability.
- **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.

KIT CONTENTS

Label	Contents 50 Columns	Contents 200 Columns
Buffer CL	25 ml	90 ml
Buffer BL[1]	25 ml	90 ml
Buffer WA[1]	40 ml	160 ml
Buffer WB[2]	10 ml	40 ml
Buffer CE[3]	20 ml	40 ml
Spin Column[4] / Collection Tube[5]	50 ea	200 ea
RNase A (Lyophilized powder)[6]	3 mg x 1 vial	3 mg x 4 vials
Proteinase K (Lyophilized powder)[6]	22 mg x 1 vial	22 mg x 4 vials

1. This buffer contains chaotropic salt.
2. ***Before use, add 40ml (160ml) of absolute EtOH to the washing buffer.***
3. DNase / RNase free Ultra-Pure solution.
4. The Columns contain silica membrane
5. Polypropylene tube for 2ml volume
6 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 ℃. These solutions are stable at -20 °C for up to 24 months and 20 times frozen-thawing until the kit expiration date.

STORAGE

G-spin™ Total DNA Extraction Kit should be stored dry at room temperature (15–25 °C). Under these conditions, G-spin™ Total DNA Extraction 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 ℃. These solutions are stable at -20 °C for up to 24 months and 20 times frozen-thawing until the kit expiration date.

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 WB
  Buffer WB is supplied as concentrate. Before using for the first time, be sure to add 40 ml (160 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 IE or distilled water for elution to room temperature.
- If Buffer CL or Buffer BL 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

G-spin™ Total DNA Extraction Kit provides almost all reagents for extracting DNA, including RNase A and Proteinase K. 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

- Equipment for disruption and homogenization, mechanical tissue grinder like pestle
- Pipettes and pipette tips
- Vortex mixer
- Microcentrifuge tubes (1.5 ml)
- Absolute ethanol (EtOH, 96~100%)
- Ice
- 1X PBS Buffer
- Water bath or heating block
- Microcentrifuge with rotor for 2.0 ml tubes
- Liquid nitrogen
- 80% ethanol
- Other general lab equipments
- Xylene Solution (for paraffin block)

QUALITY CONTROL

- In accordance with iNtRON's ISO-certified Total Quality Management System, each lot of G-spin™ Total DNA Extraction Kit is tested against predetermined specifications to ensure consistent product quality. The quality of the isolated genomic DNA was checked by restriction analysis, agarose gel electrophoresis, and spectrophotometric determination.
- G-spin™ 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.
- Buffer control : Conductivity and pH of buffers were tested and found to be within the pre-determinated ranges descrbed below.

Buffer	Conductivity	pH
Buffer CL	13.5 ~ 15.5 mS/cm	7.6 ~ 8.3
Buffer BL	120 ~ 140 mS/cm	6.9 ~ 7.6
Buffer WA	28 ~ 36 mS/cm	6.9 ~ 7.7
Buffer WB	10 ~ 12 mS/cm	7.4 ~ 8.0
Buffer CE	550 ~ 700 µS/cm	7.4 ~ 8.0

APPLICATIONS

- Cancer research
- Human genetic research
- Detection Assay : PCR, real time PCR
- DNA hybridization : Southern blotting, Microarray
- Gram negative bacterial research
- Viral DNA 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 G-spin™ Total DNA Extraction 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.

G-spin™ Total DNA Extraction 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 Departments are 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 G-spin™ Total DNA Extraction 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)

Samples	Protocol Type
Blood, Body Fluids	Type A Protocol
Tissues , Rodent tail	Type B Protocol
Cell, Buffy coat	Type C Protocol
Dried Blood Spots	Type D Protocol
Fixed Tissues	Type E Protocol
Bacteria	Type F Protocol
Biological Swabs	Type G Protocol
Animal Hair	Type H Protocol

SAMPLE PREPARATION

◈ Amounts of starting material

Use the amounts of starting material indicated in Table 2.

Table 2. Amounts of starting material for G-spin™ Total Kit procedures

Sample	Amount
Blood, plasma, serum	200 µl
Buffy coat	200 µl
Tissue	25 mg*
Cultured cells	1 x 10^6 cells
Bacterial culture (Liquid Culture)	3 OD (OD_{600})

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

Small samples should be adjusted to 200 µl with PBS before loading. For samples larger than 200 µl, the amount of Buffer CL and other reagents added to the sample before loading must be increased proportionally. Application of the lysed sample to the spin column will require more than one loading step if the initial sample volume is increased. The amounts of Buffer WA and Buffer WB used in the wash steps do not need to be increased. Scaling up the tissue protocol is possible in principle. The user should determine the maximum amount of tissue used. It is important not to overload the column, as this can lead to significantly lower yields than expected.

◈ 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 2,500 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

G-spin™ 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. Amounts of starting material for G-spin™ Total Kit procedures

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%

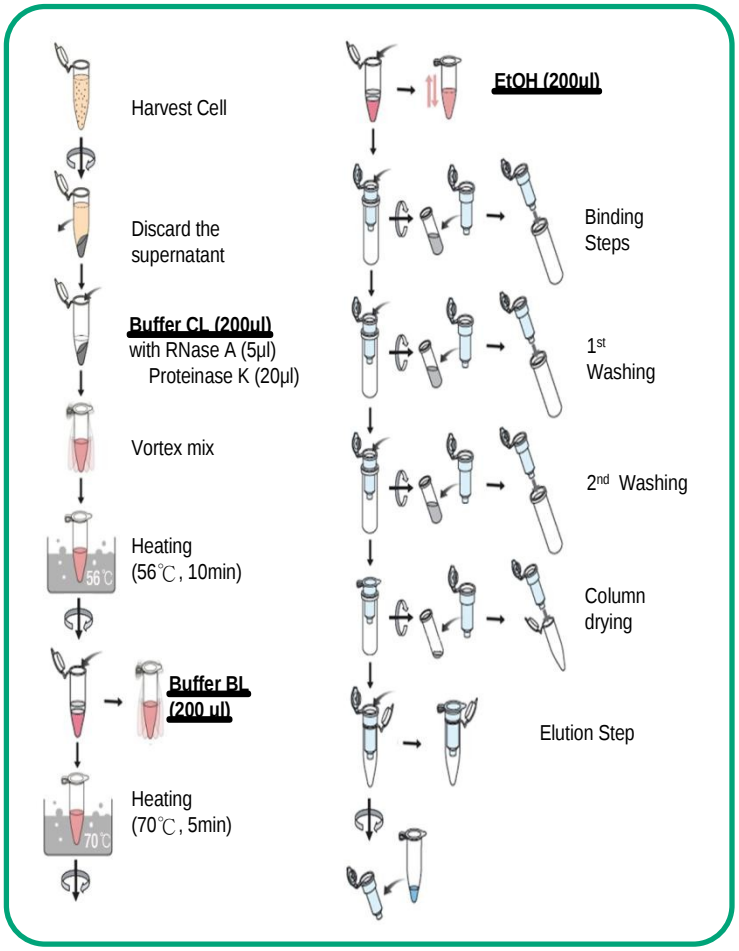
COLUMN INFORMATION

- The G-spin™ Total DNA Extraction Kit Spin Column

Column membrane[1]	Silica-based membrane
Spin Column[1]	Individually, in 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



PROTOCOL A (for Blood, body fluids)

1.Pipet 200 μl of whole blood or body fluids into a 1.5 ml microcentrifuge tube (not provided).

Note : If the volume of sample is less than 200 μl, use Buffer CL or PBS Buffer

2.Add 20 μl of Proteinase K and 5 μl of RNase A Solution 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.

3. Add 200 μl of Buffer BL 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 BL are mixed thoroughly to yield a lysis solution.

4.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.

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.

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 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 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.Add 700 μl of Buffer WA to the Spin Column without wetting the rim, and centrifuge for 1 min at 13,000 rpm. Discard the flow-through and reuse the Collection Tube.

9. Add 700 µl of Buffer WB to the Spin Column without wetting the rim, and centrifuge for 1 min at 13,000 rpm. Discard the flow-through and place the Column into a 2.0 ml Collection Tube (reuse), 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 (160) ml of absolute ethanol has been added to Buffer IWB.

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 B (for Tissue, Rodent tail)

1.Take out the target organ from laboratory animal.

Note : The fresh animal tissue can be used directly to isolation of genomic DNA. But if the tissues are not used immediately, those should be stored with liquid nitrogen (below -196 ℃) or deep freezer (below - 80℃) for long-term.

2.Slice off the prepared sample to suitable size by the scalpel or scissor.

Note : To reduce disruption and homogenization time, we recommend to slice off it. In case of enzymatic sample lysis, cut the sample 0.6 ~ 1.2 cm (mouse) or 0.3 ~ 0.6 cm (Rat) length, then slice the sample into pieces as small as possible.

3.Place the sliced sample material into a grinding jar (mortar). Add liquid nitrogen to the mortar and freeze. Keep the sample submerged in liquid nitrogen, and disrupt carefully until the sample is homogenized completely. Allow the liquid nitrogen to evaporate, and proceed immediately to step 4.

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 be difficult to lyse properly and will result in a lower yield of DNA. It's very important to keep the sample frozen in liquid nitrogen during disruption and homogenization step to inhibit low DNA yields and degraded DNA. Be careful to handle liquid nitrogen.

4. Measure 25 mg of ground tissue sample, and then transfer into 1.5 ml tube using a spatula.

Note : In order to prevent from thawing the frozen sample during transfer it, use pre-chilled the spatula and 1.5ml tube (When pre-chill the tube, the lid of tube MUST always be OPEN) with liquid nitrogen. The freeze-thaw repetition of frozen sample will result in the DNA degradation. And more, exceeding the recommended optimal amount of starting material will result in inefficient lysis, resulting in low DNA yield and purity. Ensure that the account of starting material is used, if the genomic DNA is prepared from spleen and thymus tissue, no more 10 mg should be used.

5.Add 200 µl Buffer CL, 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 co-purified with genomic DNA. RNA may inhibit downstream enzymatic reaction, but will not affect PCR.

6.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. However G-spin Total DNA Extraction Kit provides strong lysis mechanism against tissue sample. In case of cultured cell, it is enough to lysis completely for 10 ~ 15 mins, respectively. After incubation the lysate may appear viscous, but should not be gelatinous as it may clog the spin column.

7.After lysis completely, add 200 µl of Buffer BL 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.

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

Note : If insoluble tissue 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.

9.Follow the Protocol A (for Blood, body fluids) from Step 6.

PROTOCOL C (Cell, Buffy coat)

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

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.

2.Determinate the cell number using cell counter (eg. hemocytometer) and transfer the appropriated number of cells (1 ~ 3 x 10^6 cells) to a new 1.5 ml microcentrifuge tube.

3.Pellet the cell by centrifugation for 1 min at 13,000 rpm and discard the supernatant. 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.

4.Add 200 µl Buffer CL, 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 BL into upper sample tube and mix throughly. 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.Follow the Protocol A (for Blood, body fluids) from Step 6.

PROTOCOL D (for Dried Blood Spots)

1. Prepare Dried Blood Spot sample.

Note : Dried blood spot samples can be used of for performing a number of different tests. Blood sample are usually prepared from simple capillary blood samples obtained using lancets. Sometimes venous blood may be collected by needle and syringe for other purpose, in this case drawn blood can be simply spotted directly onto the collection paper. The blood is allowed to thoroughly saturate the paper and is air dried for a minimum of 3 hours. The blood collection paper is specially manufactured for blood spot. It is important that special paper be used and not substituted with regular filter paper.
There are 2 main grades of blood collection paper, 903 and 2292 (Schleicher and Schuell). It is important that all samples be collected on the same grade of paper that is used for the blood spot standard in the laboratory. These paper are manufactured from 100% pure cotton linters with no wet-strength additives. The characteristics of the blood collection paper is, Rapid absorption of blood : Single drops typically absorbed in less than ten seconds. Blood constituents easily eluted : Simply utilize water or solvent methods, depending on analyte of interest.

2. Place 3 punched-out circles from dried blood spot into 1.5ml tube.

Note : Cut 5~7 mm diameter punches from a dried blood spot with a single-hole paper puncher.

3.Add 200 µl PBS and vortex vigorously. Incubate at 85 ℃ for 10 min. Briefly centrifuge to remove drops from inside the lid.

Note : This step is pre-treating step. During the incubation time, the treated PBS solution becomes red blood color.

4. Follow the Protocol A (for Blood, body fluids) from Step 3.

PROTOCOL E (for 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 CL to the sample tube. Keep the sample submerged in Buffer CL, 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.

12.Add 150 µl Buffer CL, 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 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.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. However, G-spin™ Total DNA Extraction Mini Kit provides strong lysis mechanism against tissue sample. In case of cultured cell, it is enough to lysis completely for 15 ~ 20 min, respectively. After incubation the lysate may appear viscous, but should not be gelatinous as it may clog the spin column.

14.After lysis completely, centrifuge the sample tube at 13,000 rpm for 5 min to remove un-lysed tissue particles. Then carefully transfer 180 ~ 200 µl of the supernatant into a new 1.5 ml tube (not provided).

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

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

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

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

Note : If insoluble tissue 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.

17.Follow the Protocol A (for Blood, body fluids) from Step 6.

PROTOCOL F (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 completely 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 CL, 20 µl Proteinase K and 5 µl RNase A Solution 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 mins, respectively.

6.After lysis completely, add 200 µl of Buffer BL 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 tissue particles. Then carefully transfer 350 ~ 400 µl of the supernatant into a new 1.5 ml tube (not provided).

Note : If insoluble tissue 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 A (for Blood, body fluids) from Step 6.

PROTOCOL G (for Biological swabs)

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 ℃.

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 CL, 20 µl of Proteinase K Solution and 5 µl of RNase A into sample tube and mix vortexing vigorously. Then Incubate the lysate at 56 °C for 30 min.

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

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

5.Add 400 µl of Buffer BL into the lysate, and mix well by gently inverting 5 - 6 times. After mixing, incubate the lysate at 70°C for 5 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 2 ml Collection Tube (reuse).

10. Follow the Protocol A (for Blood, body fluids) from Step 8

PROTOCOL H (for Animal hair)

1.Prepare 10 pieces of hair.

Note : In the case of hair sample, the purification result is changed greatly according to existence or nonexistence of hair root. If it is possible, it is desirable to use sample which contains hair root. In case of no hair root sample, it is available to detect the result only PCR amplification

2.Cut the hair sample from hair root 1 cm length, then carefully transfer the sample into a new 1.5 ml tube.

Note : Carefully handle to sample without loss of hair root because the hair root tends to adhesive on surface of solid material.

3.Crash the hair sample 10 ~ 20 times using micro-pestle with 50 µl of Buffer CL.

Note : The hair is not solubilized in Buffer CL, But hair root part is soluble in Buffer CG. It is difficult too small to estimate in the unaided whether the sample is homogenized well or not. Thus, crash the sample 10 ~ 20 times using micro-pestle.

4.Add 150 µl Buffer CL (include that use in homogenizing step), 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 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.

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.

6.After lysis completely, add 200 µl of Buffer BL 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 tissue particles. Then carefully transfer 350 ~ 400 µl of the supernatant into a new 1.5 ml tube (not provided).

Note : If insoluble tissue 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 A (for Blood, body fluids) from Step 6.

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 BL	• Repeat the DNA purification procedure with a new sample. Be sure to mix the sample and Buffer BL 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 BL
	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 BL	• Repeat the DNA purification procedure with a new sample. Be sure to mix the sample and Buffer BL immediately and thoroughly by pulse-vortexing.
	Inefficient cell lysis or protein degradation in Buffer CL or Buffer BL 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. Ensure that no residual particulates are visible

TROUBLESHOOTING GUIDE

Problem	Possible Cause	Recommendation
Little or no DNA in the eluate	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.
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 WA and WB used in the wrong order	• Ensure that Buffers WA and WB 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 be formed after storage at low temperature or prolonged storage	• Any precipitate in Buffer CL or Buffer BL 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. • Plasma samples: Cryoprecipitates have formed in plasma due to repeated freezing and thawing. Do not use plasma that has been frozen and thawed more than once.
	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.

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.

◈ 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.

EXPERIMENTAL INFORMATION

◈ Recovery Table

The genomic DNA extraction results using G-spin™Total DNA Extraction Kit was shown high quality and quantity of DNA collected from cultured animal cells, blood, tissue samples, gram-negative bacteria and biological swab samples.

Table 4. Determination of yield and purity data of various samples

Sample	type	Lane	Amount	DNA yield (μg)	A260/280
Cell	K562	1	10^6 cells	7 ~ 20	1.9 ±0.2
	SNU-1	2	10^6 cells	7 ~ 20	1.9 ±0.2
	U937	3	10^6 cells	7 ~ 20	1.9 ±0.2
	HeLa	4	10^6 cells	7 ~ 20	1.9 ±0.2
	NIH3T3	5	10^6 cells	7 ~ 20	1.9 ±0.2
	Vero	6	10^6 cells	7 ~ 20	1.9 ±0.2
	B16	7	10^6 cells	7 ~ 20	1.9 ±0.2
Tissue	Liver (mouse)	8	25mg	10 ~ 20	1.9 ±0.2
	Heart (mouse)	9	25mg	8 ~ 16	1.9 ±0.2
	Lung (mouse)	10	25mg	8 ~ 16	1.9 ±0.2
	Brain (mouse)	11	25mg	10 ~ 20	1.9 ±0.2
	Kidney (mouse)	12	25mg	10 ~ 20	1.9 ±0.2
	Spleen (mouse)	13	10mg	9 ~ 20	1.9 ±0.2
	muscle (mouse)	14	25mg	3 ~ 6	1.9 ±0.2
	stomach (mouse)	15	25mg	5 ~ 10	1.9 ±0.2
	Tail (mouse)	16	25mg	12 ~24	1.9 ±0.2
	Hair (human)	17	10 ea	10 ~ 20	1.9 ±0.2
Blood	blood (EDTA)	18	200μl	4 ~ 10	1.9 ±0.2
	blood (Heparin)	19	200μl	4 ~ 10	1.9 ±0.2
	blood (citrate)	20	200μl	4 ~ 10	1.9 ±0.2
	Buffy coat 1	21	from 300μl	6 ~ 12	1.9 ±0.2
	Buffy coat 2	22	from 300μl	6 ~ 12	1.9 ±0.2
Bacteria	*E. coli*	*23*	3OD	8 ~ 16	1.9 ±0.2
	P. aeruginosa	*24*	3OD	7 ~ 14	1.9 ±0.2
	S. gallinarium	*25*	3OD	8 ~ 16	1.9 ±0.2
Swab	Blood swab	26	1ea	2 ~ 5	1.9 ±0.2
	Buccal swab	27	1ea	5 ~ 15	1.9 ±0.2
Fixed Tissue	Liver (formalin)		25mg	< 1	ND
	Liver (paraffin)		25mg	< 1	ND

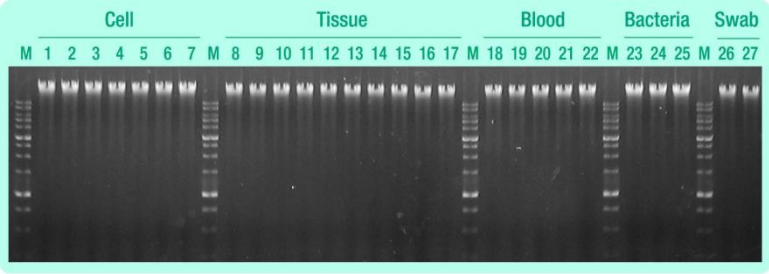


Fig. 1. Agarose gel electrophoresis of extracted genomic DNA from various samples

Lane M, SiZer™-1000 plus DNA Marker; **Lane 1 ~ 27**, refer to lane information of table 4.

◈ Comparative Test of gDNA extraction efficiency with competitive products

A. Case 1 : Whole blood samples

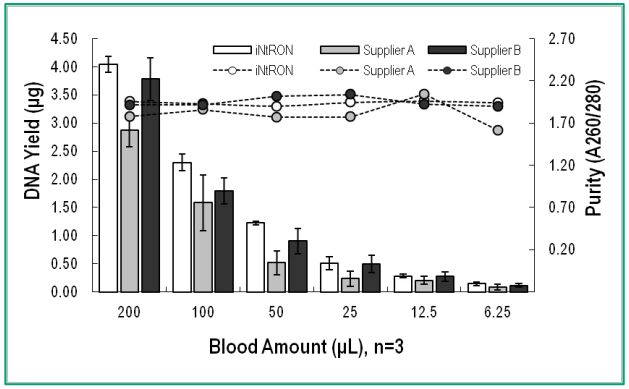


Fig 2. Comparison of DNA Yields of different amount of blood sample with Competitor's

The whole blood samples were used from 6.25μl to 200μl. The yields of blood genomic DNA were proportionally increased with blood starting amount. G-spin™ Total DNA Extraction Kit shows improved efficiency of DNA extraction from whole blood samples.

B. Case 2 : Cultivated cell and bacteria samples

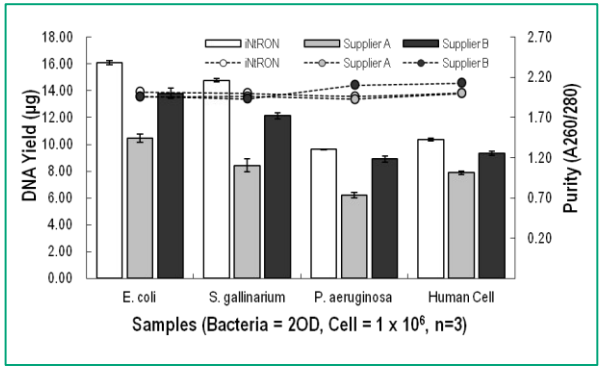


Fig 3. Comparison of DNA Yields of different amount of cell sample with Competitor's

The cultivated cell (gram negative bacteria and human cell) samples were used from 2 OD (bacteria) and 1 x 10 ^{6} cells (human cell). The G-spin™ Total DNA Extraction Kit shows improved efficiency of DNA extraction from cultivated cell samples.

C. Case 3 : Tissue samples

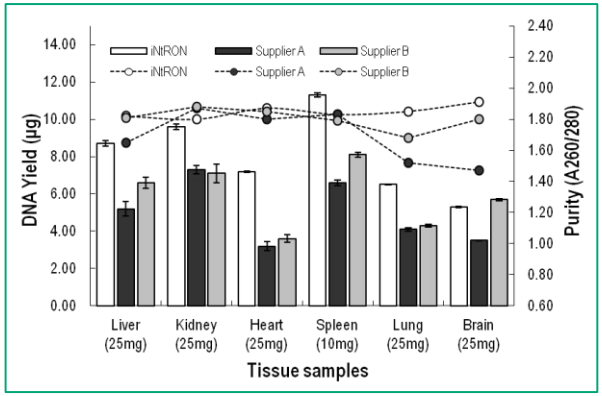


Fig 4. Comparison of DNA Yields of various tissue sample with Competitor's

The tissue samples were used from 10 to 25mg. The G-spin™ Total DNA Extraction Kit shows improved efficiency of DNA extraction from tissue samples.

◈ **High quality and quantity of recovered DNA**

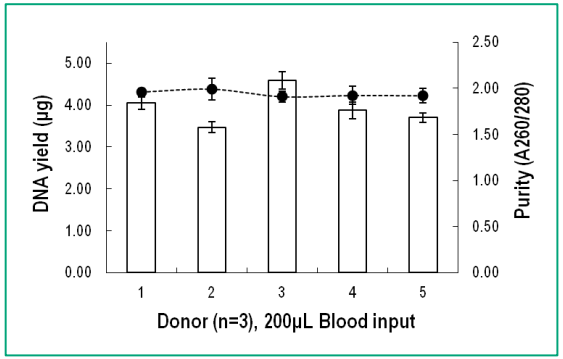


Fig 5. G-spin™ Total DNA Extraction Kit delivers buffy coat-like yield and concentration from whole blood.

DNA yield and concentration from blood samples was obtained from 5 individual donors (200μl of whole blood was processed; 3 replicates were performed from each donor).

◈ **Improved efficiency compared to previous product**

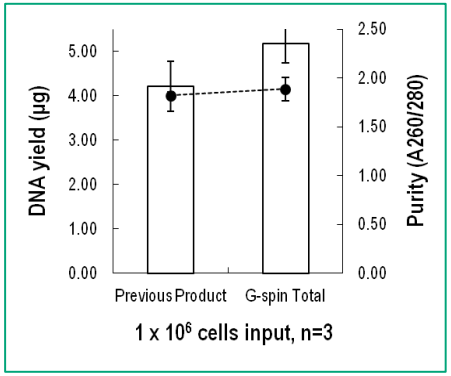


Fig 6. Comparison of DNA Yields of blood sample with previous product.

The whole blood samples were used from 200μl. The yields of blood genomic DNA were increased to 15 ~ 25 % better than the older product.