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.

RUO *Research Use Only*

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DESCRIPTION

- G-spin™ Total DNA Extraction Mini Kit provides 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 sample for reliable PCR and Southern blotting. Furthermore, we have tested G-spin™ Total DNA Extraction Mini Kit to get more practical data from various biological samples.
- The simple G-spin™ Total DNA Extraction protocols, which are ideal for simultaneous processing of multiple samples, yield pure DNA to be prepared for direct amplification within 20 ~ 30 minutes. The G-spin™ Total DNA Extraction Mini Kit 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 does not require phenol/chloroform extraction or EtOH precipitation, and provides the simplest protocols. DNA is eluted in Buffer CE , TE (10:1), 10mM Tris (pH 7.5 ~ 8) or water, is prepared for direct addition to PCR or other enzymatic reactions. Alternatively, it can be safely stored at –20°C for later use. The purified DNA is protein-free, nucleases-free and does not include other contaminants or inhibitors. G-spin™ Total DNA Extraction Mini Kit is optimized for extraction of 20-30kb DNA fragments and able to extract up to 50 kb fragments.
- G-spin™ Total DNA Extraction Mini Kit provides various protocols. You can also extract genomic DNA from various biological samples by selecting an appropriate protocol from Protocol list. If you need some more information on selecting a protocol, please do not hesitate to contact our Technical Assistance Team.

CHARACTERISTICS

- **Fast** : 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. Freeze-dried formulated enzyme has been improved DNA extraction stability.
- **Advantage** : The Kit is suitable for 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]	14 ml	56 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 56ml (224ml) 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's expiration date. 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's 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. The lyophilized RNase A and Proteinase K can be stored at room temperature (15–25°C) until the kit's expiration date without affecting activity. 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's expiration date.

APPLICATIONS

- Cancer research
- Human genetic research
- Detection Assay : PCR, real time PCR
- DNA hybridization : Southern blotting, Microarray
- Gram negative bacterial research
- Viral DNA Research

PROTOCOL LIST

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, Bacteria	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

- Version upgraded from conventional protocol
- Proceed as with existing protocol

ADDITIONAL REQUIRED EQUIPMENT

G-spin™ Total DNA Extraction Kit provides almost all reagents for extracting DNA, including RNase A and Proteinase K. However, be prepared some equipments and reagents as follows for a fast and easy extraction.

- Equipment for disruption, homogenization and 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)

SAFETY INFORMATION

All chemicals should be considered as potentially hazardous. When handling 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.

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 DNA recovery of G-spin™ column for 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 described below.

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

PRODUCT WARRANTY AND SATISFACTION GUARANTEE

All products undergo extensive quality control test and are warranted to perform as described when used correctly. Satisfaction guarantee is conditional upon the customer providing full details of the problem to iNtRON within 60 days of purchase, 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 use only. They are not to be used for human or animal diagnostics. Do not use internally or externally in humans or animals. Be careful to handle product.

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 affect DNA binding capacity. The Spin Columns are stable for over 2 year under these conditions

CONSIDERATION BEFORE USE

- **Lyophilized RNase A** : Dissolve the RNase A in 0.3 ml of pure D.W.
- **Lyophilized Proteinase K :** Dissolve the Proteinase K in 1.1 ml of pure D.W.

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

- Preheat a water bath or heating block
- 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)

SAMPLE PREPARATION

◈ Amounts of starting material

Use the amounts of starting material indicated in Table (below).

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 the loading increasis 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 washing 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.

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

Storage of DNA

For long-term storage, DNA should be dissolved in buffer TE or Buffer CE and stored at –20°C. Any contaminants in the DNA solution may lead to DNA degradation. Avoid repetitive freezing-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 number of cuvettes must be matched to the number of samples.

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

Note : Phenol has a maximum absorbance at 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 and contaminants such as protein which absorbs UV light. 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 adjust spectrometer at zero using 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.

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 ensure 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 : In order to ensure efficient lysis, it is important that the blood sample and Buffer BL are mixed thoroughly to yield a lysis solution.

4. **Place the mixture at Room Temperature for 2minutes.**

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 lid.**

7. **Add 200 µl of absolute ethanol into the lysate, and mix well by pulse 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 WA (Buffer WB) 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.**
   Note : Use Buffer WB for cell sample.

10. **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 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.

11. **Place the Spin Column into a new 1.5 ml tube (not supplied), and add 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 : In general, Elution with 30 µl (instead of 50 µl) increases the final DNA concentration, but reduces overall DNA yield.
    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℃).

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 to 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. 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 type of tissue samples. We recommend samples to be disrupted completely until tissue clumps are not shown. Clumps of tissue sample will be difficult to lyse properly and will result in a low 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 DNA degradation. 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 frozen sample from thawing, use pre-chilled spatula and 1.5ml tube (When pre-chill the tube, the lid of tube MUST always be OPEN) with liquid nitrogen during transferring. The freezing-thawing repetition of frozen sample will result in the DNA degradation. Furthermore, exceeding the recommended optimal amount of starting material will result in inefficient lysis, resulting in low DNA yield and purity. The amount of starting material should be measured. If the genomic DNA is prepared from spleen and thymus tissue, no more than 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, contains 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℃ using 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 cells, 10~15 min is enough to lysis completely. After incubation, the lysate may appear viscous, but should not be gelatinous as it may clog the spin column.

7. **When lysis is completed, add 200 µl of Buffer BL into upper sample tube and mix thoroughly. Then incubate the mixture at 70℃ for 5min.**
   Note : Vigorous vortexing may 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 remain in homogenated mixture, spin column clogging will occur sometimes. This step helps sample mixing with buffer during binding step. 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 to mix the cell pellet and remnant supernatant 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. **After mix, add 200ul of Buffer BL to upper sample tube and mix throughly.**

6. **Place the mixture at Room Temperature for 2minutes.**

7. **Incubate the lysate at 56℃ (preheated heat block or water bath) for 10 min.**
   Note : To assist 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.

8. **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 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 of 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) 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 (no 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 paraffin as much 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 : Removal of the remnant ethanol 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 adding buffer. After preliminary crashing, add 50 µl of Buffer CL to the sample tube. Keep the sample submerged in Buffer CL, and disrupts carefully until the sample is homogenized completely.

Note : Disruption and homogenization time depends on the type of tissue samples. We recommend samples to be disrupted completely until tissue clumps are not shown. Clumps of tissue sample will be difficult to lyse properly and result in a low 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℃ using preheated heat block or water bath for 10 ~ 30 min.

Note : To assist 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 cells, 10~15 min is enough to lysis completely. After incubation, the lysate may appear viscous, but should not be gelatinous as it may clog the spin column.

14.When lysis is completed, 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 remain in homogenated mixture, spin column clogging will occur sometimes. This step helps sample mixing with buffer during binding step. Also, It prevents column clogging from insoluble clumps.

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

Note : Vigorous vortexing may induce genomic DNA breakage. In order to ensure 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 remain in homogenated mixture, spin column clogging will occur sometimes. This step helps sample mixing with buffer during binding step. 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 amount of starting material is used 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 to mix the pellet and remnant supernatant 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℃).

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

Note : To assist 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.

6. When lysis is completed, add 200 µl of Buffer BL into upper sample tube and mix thoroughly. Then incubate the mixture at 70℃ for 5min.

Note : Vigorous vortexing may 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 remain in homogenated mixture, spin column clogging will occur sometimes. This step helps sample mixing with buffer during binding step. 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 °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 CL Buffer, 20 µl of Proteinase K Solution and 5 µl of RNase A into sample tube and mix by 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°C).

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

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 lid.**

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 ensure proper mixing after adding the ethanol, until not showing 2-phase which is not mixed. Also, this step conduces cell lysate to pass efficiently 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.**

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 case of hair sample, the purification result is changed greatly according to existence or nonexistence of hair root. If 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 by 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 the 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 : Unlike hair root part, hair is not solubilized in Buffer CL. Make sure samples should be crashed 10 ~ 20 times using micro-pestle.

4. **Add 150 µl of Buffer CL, 20 µl of Proteinase K and 5 µl of 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℃ using preheated heat block or water bath for 10 ~ 30 min.**
   Note : To assist lysis tissue sample, mix the tube by inverting every 2 min during the incubation. Lysis time varies depending on the type of sample.

6. **When the lysis is completed, add 200 µl of Buffer BL into upper sample tube and mix thoroughly, then incubate the mixture at 70℃ for 5min.**
   Note : Vigorous vortexing may induce genomic DNA breakage. In order to ensure 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 remain in homogenated mixture, spin column clogging will occur sometimes. This step helps sample mixing with buffer during binding step. 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 by 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 according to the problem occurred in Buffer CL or Buffer BL	• 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 precipitates 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.
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
	WA and WB Buffers used in the wrong order	• Ensure that WA and WB Buffers 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 buffers at 56°C. The precipitate has no effect on kit's 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 per 200 µl. Dilute the sample with PBS and repeat the purification. • Plasma samples: Cryoprecipitates have formed in plasma due to repetitive 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.

EXPERIMENTAL INFORMATION

◈ DNA Recovery from different amount of blood samples

The iNtRON's G-spin™ Total DNA Extraction Kit gave improved DNA yield compare with competitor's. from various amount of total blood samples.

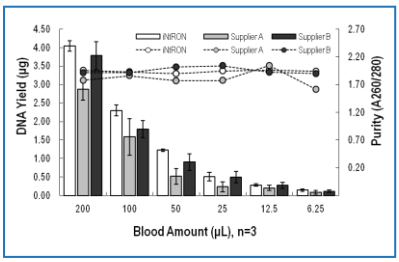

Fig 1. Comparison of DNA Yields of different amount of blood sample with Competitor's

The whole blood samples were used from 6.25μl to 200μl. The yields of blood genomic DNA were increased proportionally with blood start amount.

G-spin™ Total DNA Extraction Kit shows improved efficiency of DNA extraction from whole blood samples.

◈ High quality and quantity of recovered DNA

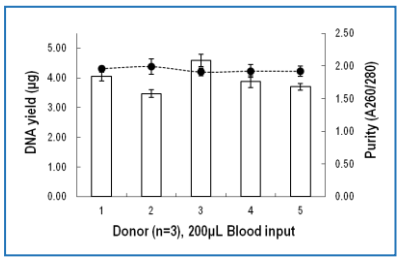

Fig 2. G-spin™ Total DNA Extraction Kit delivers buffy coat-like yield and concentration from whole blood.

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)..

◈ Recovery Table

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

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

Quick Guide