G-spin™ Total DNA Extraction Kit

QUICK GUIDE

50 prep : 17045
200 prep : 17046

1. KIT DESCRIPTION

G-spin™ Total DNA Extraction Kit provides a fast, efficient, and reliable silica-membrane method for the isolation of total genomic DNA from a wide variety of biological samples, including whole blood, body fluids, animal tissues, cultured cells, bacterial cultures, and swab specimens. The advanced spin-column technology eliminates the need for phenol/chloroform extraction or alcohol precipitation, yielding pure nucleic acids ready for direct downstream amplification within 20–30 minutes. The purified DNA is free of protein, nucleases, and enzymatic inhibitors, making it highly suitable for molecular diagnostic research and veterinary testing platforms.

2. CHARACTERISTICS

- **Broad Sample Versatility** : High recovery efficiency across whole blood, buffy coat, tissues, rodent tail, cultured cells, Gram-negative bacteria, swabs, dried blood spots, FFPE, and animal hair.
- **High Yield & Purity** : Delivers intact genomic DNA (up to 50 kb, centered at 20–30 kb) with an A_{260}/A_{280} ratio of 1.7–1.9.
- **Complete Inhibitor Removal** : Efficiently eliminates PCR inhibitors (heparin, hemoglobin, salts) to ensure high analytical sensitivity.
- **Lyophilized Enzyme Formulation** : Pre-measured, lyophilized Proteinase K and RNase A guarantee maximum enzymatic stability and long-term activity.

3. INTENDED USE & APPLICATIONS

- **Intended Use :** This product is intended for professional use in molecular diagnostic research and veterinary in vitro diagnostic (IVD) workflows to extract genomic DNA for target detection and surveillance.
- **Applications :** Suitable for downstream molecular assays including PCR, real-time PCR (qPCR), Southern blotting, microarray analysis, and NGS library preparation.

4. KIT CONTENTS & STORAGE

Contents	17045 (50 preps)	17046 (200 preps)
Buffer CL	25 ml	90 ml
Buffer BL	25 ml	90 ml
Buffer WA	40 ml	160 ml
Buffer WB* (concentrate)	10 ml (add 40ml EtOH)	40 ml (add 160ml EtOH)
Buffer CE	20 ml	40 ml
Spin Columns (with collection tubes)	50 ea	200 ea
Proteinase K (Lyophilized)**	22mg x 1 tube	22mg x 4 tubes
RNase A (Lyophilized)**	3mg x 1 tube	3mg x 4 tubes

- Storage & Stability: Store all liquid buffers and spin columns at room temperature (15–25ºC). The kit is stable for up to 24 months under recommended conditions.
- Documentation: Quick Guide included.

* Buffer WB Preparation : Add absolute ethanol (96–100%) to Buffer WB concentrate before initial use
- For Cat. 17045 (50 preps): Add 40 ml of absolute EtOH.
- For Cat. 17046 (200 preps): Add 160 ml of absolute EtOH.

** Enzyme Dissolution & Storage :
- Dissolve Proteinase K in 1.1 ml sterile D.W.
- Dissolve RNase A in 0.3 ml sterile D.W.
- Store reconstituted enzymes at -20ºC. Stable for up to 24 months and 20 freeze-thaw cycles

✱ Additional Required Materials

- **Reagents :** Absolute ethanol (96–100%) | PBS Buffer
- **Equipments :** Vortex mixer | Microcentrifuge | Heating block | Pipettes & nuclease-free tips
- **For special samples :** Xylene (FFPE), Micro-pestle (Hair/Tissue), Paper puncher & DBS collection paper (DBS).

5. SAFETY INFORMATION & HAZARD WARNINGS

- **Personal Protective Equipment (PPE) :** All chemical components should be handled with standard laboratory safety precautions. Always wear appropriate personal protective equipment, including disposable nitrile gloves, a chemical-resistant laboratory coat, and protective eyewear, when handling samples and kit reagents.
- **Chemical Hazard & Toxic Gas Release Warning :** Buffer WA and Buffer BL contain chaotropic agents (guanidine salts).

  WARNING : DO NOT add bleach (sodium hypochlorite) or acidic solutions directly to the sample preparation waste or effluents containing these buffers. Reaction of chaotropic salts with bleach or acids generates highly toxic, corrosive, and lethal gases (e.g., hydrogen cyanide, chlorine gas).

- **Flammability Hazard :** Buffer WB (after ethanol addition) and absolute ethanol solutions are flammable. Keep away from open flames, heat sources, sparks, and hot surfaces. Do not smoke in reagent handling areas.
- **Decontamination & Waste Disposal :** If liquid spills occur, clean immediately with detergent and water. If liquid containing potential biohazards/infectious agents is spilled, clean first with detergent and water, then treat with an appropriate disinfectant. Dispose of chemical and biological liquid waste in accordance with local, regional, and national biosafety and hazardous materials regulations.

6. BEFORE YOU BEGIN

- **Column Specifications**
  - **Membrane** : Silica-based membrane
  - **Max. Loading Volume** : 800 µl
  - **Max. Binding Capacity** : 45 µg gDNA
  - **Recommended Elution Volume** : 30 – 200 µl
- **General Rule & Pre-handling**
  - **Pre-heating** : Set heating blocks to 56ºC and 70 ºC prior to starting.
  - **Precipitates in Buffers** : If Buffer CL or BL forms precipitates during low-temperature storage, redissolve by incubating at 56ºC with gentle agitation.
  - **Sample Input Limits** : Do not overload the column (max 25 mg general tissue, max 10 mg spleen/thymus. Overloading reduces yield and clogs the membrane.
- **Buffer WB Preparation** :

  Note : Add absolute ethanol (96–100%) to Buffer WB concentrate before initial use
  - 50 preps (Cat. 17045) : Add 40ml of absolute EtOH.
  - 200 preps (Cat. 17046) - Add 160 ml of absolute EtOH.

- **Enzyme Reconstitution :**

  Note : Reconstitute lyophilized Proteinase K in 1.1 ml sterile D.W., and RNase A in 0.3 ml sterile D.W. Aliquot & store at -20 ºC.

- **Equipment Setup** : Preheat water baths or heating blocks to 56 ºC, 70 ºC, and 85 ºC as required for target sample types.

7. PROTOCOLS

7.1. SAMPLE PRE-TREATMENT WORKFLOW (BY SAMPLE MATRIX)

A. Whole Blood, Buffy Coat & Body Fluids

1a) Whole Blood / Body Fluids : Pipette 200 µl of whole blood or body fluid sample (fresh or frozen treated with EDTA, Citrate, or Heparin) into a 1.5 ml microcentrifuge tube.
Note : Standard input is 200 µl. If sample volume is <200 µl, adjust total volume to 200 µl using PBS or Buffer CL.

1b) Buffy Coat : Prepare 200 µl of buffy coat fraction (isolated from up to 300 µl whole blood) in a 1.5 ml tube.

2) Enzyme & Lysis Addition: Add 20 µl Proteinase K, 5 µl RNase A, and 200 µl Buffer BL. Pulse-vortex thoroughly.
Note : Thorough mixing of sample and Buffer BL is critical to yield a homogeneous lysis solution.

3) Incubation: Incubate at 56°C for 10 min.
Note : Invert the tube 3–4 times during incubation. Perfect lysis turns whole blood from red to dark green.

4) Binding Preparation : Add 200 µl absolute EtOH and pulse-vortex until fully mixed without phase separation. → Proceed to Section 7.2 Core Purification Workflow.

B. Animal Tissues & Rodent Tail

1) Sample Preparation & Lysis: Place 25 mg of sliced tissue into a 1.5 ml tube, then add 200 µl Buffer CL, 20 µl Proteinase K, and 5 µl RNase A. Vortex vigorously.
Note : Standard input is 25 mg for general tissue or rodent tail (mouse: 0.6–1.2 cm / rat: 0.3–0.6 cm). For high cell-density tissues such as spleen or thymus, do not exceed 10 mg. Pre-chilling tools or slicing tissue into minimal pieces significantly reduces lysis time.)

2) Digestion : Incubate at 56°C for 10–30 min (invert tube every 2 min) until tissue is fully dissolved.
Note : Lysis duration varies by tissue type. The lysate should appear viscous but not gelatinous.

3) Secondary Lysis : Add 200 µl Buffer BL, mix thoroughly, and incubate at 70°C for 5 min.

4) Clarification: Centrifuge at 13,000 rpm for 5 min. Carefully transfer 350–400 µl of clear supernatant to a clean tube.
Note : Removing un-lysed tissue debris prevents spin column clogging.

5) Binding Preparation : Add 200 µl absolute EtOH and mix well by pulse-vortexing. → Proceed to Section 7.2 Core Purification Workflow.

C. Cultured Cells & Gram-Negative Bacteria

1a) Cultured Cells: Pellet 1–3x10^6 cells, wash with PBS, and completely remove supernatant. Resuspend cell pellet completely in 200 µl Buffer CL.
Note : Standard starting amount is 1x10^6 to 3x10^6 cells.

1b) Gram-Negative Bacteria: Centrifuge 1–2 ml of bacterial liquid culture (OD600 = 0.8–1.0, equivalent to 3 OD) at 13,000 rpm for 1 min. Discard supernatant completely and resuspend pellet in 200 µl Buffer CL.
Note : Do not exceed 3 OD input as excess starting material leads to incomplete lysis and low purity.

2) Enzyme & Lysis Addition: Add 20 µl Proteinase K, 5 µl RNase A, and 200 µl Buffer BL. Pulse-vortex thoroughly.
Note : Thorough mixing of sample and Buffer BL is critical to yield a homogeneous lysis solution.

3) Incubation: Incubate at 56°C for 10 min.
Note : Invert the tube 3–4 times during incubation.

4) Binding Preparation: Add 200 µl absolute EtOH and mix thoroughly. → Proceed to Section 7.2 Core Purification Workflow.

D. Dried Blood Spots (DBS)

1) Pre-treatment: Punch 3 discs (5–7 mm diameter) from a dried blood spot into a 1.5 ml tube. Add 200 µl PBS, vortex, and incubate at 85°C for 10 min.
Note : Standard input is 3 punched-out circles (5–7 mm diameter) collected on standard blood collection paper (e.g., 903 or 2292). During incubation, the PBS solution will turn red.

2) Enzyme & Lysis Addition: Add 20 µl Proteinase K, 5 µl RNase A, and 200 µl Buffer BL. Incubate at 56°C for 10 min.

3) Binding Preparation: Add 200 µl absolute EtOH and mix well. → Proceed to Section 7.2 Core Purification Workflow.

E. Fixed Tissues (FFPE / Formalin Fixed)

1) Deparaffinization : Place sliced tissue (≤ 25 mg) into a tube. Add 1.2 ml Xylene, vortex, centrifuge at 13,000 rpm for 5 min, and discard supernatant without disturbing pellet (repeat once).
Note : Maximum starting amount is 25 mg of fixed tissue (≤ 10 mg for spleen/thymus). Ensure paraffin is removed as much as possible to maximize yield.

2) Wash & Dry : Wash pellet twice with 1.2 ml absolute EtOH. Incubate open tube at 65°C for 10–15 min until ethanol is completely evaporated.
Note : Complete ethanol removal during deparaffinization is essential for successful lysis.) Lysis & Digestion: Follow Category B (Animal Tissues) Steps 1 through 4.

3) Binding Preparation: Add 200 µl absolute EtOH and mix well. → Proceed to Section 7.2 Core Purification Workflow.

F. Biological Swabs

1) Pre-treatment & Primary Lysis : Place 1 swab head (Cotton or DACRON) in a 1.5 ml tube. Add 400 µl Buffer CL, 20 µl Proteinase K, and 5 µl RNase A. Incubate at 56°C for 30 min.
Note : Standard input is 1 biological swab head. Cut swab stick using clean scissors.

2) Secondary Lysis: Add 400 µl Buffer BL, mix, and incubate at 70°C for 5 min.

3) Binding Preparation: Add 400 µl absolute EtOH and mix gently by inversion 5–6 times (DO NOT vortex).

4) Column Loading: Apply 800 µl of mixture sequentially onto the spin column. → Proceed to Section 7.2 Core Purification Workflow.

G. Biological Swabs

1) Disruption: Cut 10 hair pieces (1 cm length, preferably with hair roots) into a tube. Grind hair 10–20 times using a micro-pestle in 50 µl Buffer CL.
Note : Standard input is 10 hair pieces (1 cm length). Hair roots yield significantly higher DNA recovery. Hair shafts do not solubilize in Buffer CL; physical disruption via micro-pestle is mandatory.

2) Secondary Lysis: Add 400 µl Buffer BL, mix, and incubate at 70°C for 5 min.

3) Binding Preparation: Add 400 µl absolute EtOH and mix gently by inversion 5–6 times (DO NOT vortex).

4) Column Loading: Apply 800 µl of mixture sequentially onto the spin column. → Proceed to Section 7.2 Core Purification Workflow.

7.2. CORE PURIFICATION WORKFLOW

(Applicable to all pre-treated sample lysates from Section 7.1)

1) **Column Loading : Transfer the prepared lysate/ethanol mixture onto a Spin Column pre-inserted in a 2.0 ml Collection Tube. Centrifuge at 13,000 rpm for 1 min. Discard flow-through and reinsert the column.**
Note : Close column caps during centrifugation to prevent aerosol formation. Do not transfer heavy solid particulates.

2) **First Wash : Add 700 µl Buffer WA to the spin column without wetting the rim. Centrifuge at 13,000 rpm for 1 min. Discard flow-through and reinsert column.**

3) **Second Wash : Add 700 µl Buffer WB (ethanol added) to the spin column. Centrifuge at 13,000 rpm for 1 min. Discard flow-through and reinsert column.**

4) **Membrane Drying : Centrifuge at 13,000 rpm for an additional 1 min to completely dry the membrane.**
Note : Complete removal of residual ethanol is essential. Residual ethanol inhibits downstream PCR/qPCR amplification.

5) **Elution : Transfer column to a clean, RNase-free 1.5 ml tube. Add 30–100 µl Buffer CE directly onto the center of the silica membrane. Incubate at RT for 1 min, and centrifuge at 13,000 rpm for 1 min to elute pure gDNA.**
Note : Avoid touching the silica membrane with the pipette tip. Elution with lower volumes, e.g., 30 µl, yields higher DNA concentration but slightly lower total recovery.

8. TROUBLESHOOTING GUIDE

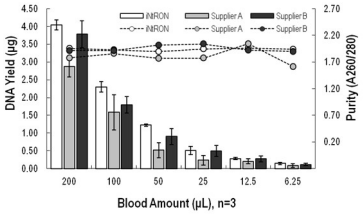
Problem	Possible cause	Recommendation
Low DNA yield	Incomplete cell or tissue lysis	Ensure immediate and thorough mixing with Buffer BL. Extend the 56°C digestion time and verify Proteinase K activity. Ensure sample is finely sliced.
	Omission of absolute ethanol	Ensure absolute ethanol (96–100%) was added to the lysate prior to spin column loading. Do not use denatured alcohol containing methanol or MEK.
	Overloaded sample input	Do not exceed recommended starting material limits (e.g., max 25 mg tissue, max 10 mg spleen/thymus). Overloading leads to incomplete digestion and column clogging.
Low purity ratio (A260/A280 < 1.7)	Protein contamination	Re-treat lysate with freshly reconstituted Proteinase K and ensure full incubation at 56°C. Do not add Proteinase K directly into Buffer BL.
	Insufficient wash step	Ensure Buffer WA and Buffer WB are used in the correct sequential order.
Low purity ratio (A260/A230 < 1.5)	Chaotropic salt or ethanol carryover	Ensure complete washing with Buffer WB. Perform an extra drying centrifugation step at 13,000 rpm for 1–2 minutes to remove all residual salts and ethanol.
Spin column clogging / Slow flow	Insoluble tissue debris or excessive cell input	Centrifuge the lysate at 13,000 rpm for 5 minutes after digestion and transfer only the clear supernatant to the column. If using blood with high leukocyte count (>5x10^5 per 200 µl), dilute sample with PBS before processing.
	Cryoprecipitates in plasma	Avoid using plasma samples that have undergone multiple freeze-thaw cycles.
DNA degradation / Sheared DNA	Vigorous vortexing after lysis	Avoid excessive or violent vortexing after Buffer BL addition to prevent physical shearing of high-molecular-weight genomic DNA.
	Repeated freeze-thaw of sample	Avoid repetitive freezing and thawing of starting samples or eluted gDNA. Store purified DNA aliquots at -20°C.
RNA contamination	Ineffective RNase A digestion	Ensure RNase A was added during the initial lysis step. Verify that RNase A stock solution was reconstituted and stored correctly at -20°C.
Inhibited downstream PCR/qPCR	Residual ethanol carryover	Ensure the spin column membrane is completely dried by centrifuging at 13,000 rpm for an additional 1 minute before elution.
	Carryover of PCR inhibitors	For blood samples containing heavy heparin, or swab samples with high debris, increase the elution volume or dilute the eluate 1:5 to 1:10 prior to PCR setup.
Precipitate in Buffers (Buffer CL or BL)	Low storage temperature	Incubate Buffer CL or Buffer BL at 56°C with gentle shaking until completely redissolved. Precipitates do not compromise buffer performance once redissolved.

9. EXPERIMENTAL INFORMATION

※ Experimental Data 1 : DNA Recovery Across Variable Blood Sample Volumes

The iNtRON G-spin™ Total DNA Extraction Kit demonstrated significantly improved DNA yields compared with competitor kits across various input volumes of whole blood samples.

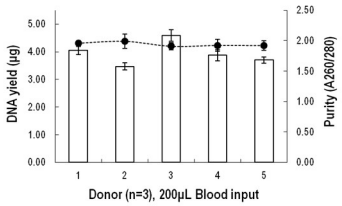
Fig 1. Comparison of DNA Extraction Yields Across Variable Blood Input Volumes Against Competitors



Whole blood samples ranging from 6.25 µl to 200 µl were processed. DNA recovery increased proportionally with increasing starting blood volume. The G-spin™ Total DNA Extraction Kit exhibits superior extraction efficiency and linearity across all tested whole blood volumes.

※ Experimental Data 2 : High Quality and Quantity of Recovered Genomic DNA

Fig 2. Consistent Yield and High Purity of Genomic DNA Extracted from Whole Blood



Genomic DNA yield and concentration were evaluated across 5 individual human donors (200 µl whole blood per prep; n=3 replicates per donor). The G-spin™ Total DNA Extraction Kit reliably delivers high-purity, buffy coat-equivalent yields directly from whole blood.

※ Recovery : Extraction Performance Across Diverse Sample Matrices

Genomic DNA extraction using the G-spin™ Total DNA Extraction Kit demonstrates high yield and consistent purity (A260/280 = 1.9 ± 0.2) across a wide range of biological matrices, including cultured animal cells, whole blood, tissue samples, Gram-negative bacteria, and biological swabs.

Table 1. Summary of Representative DNA Yield and Purity

Sample Category	Representative Sample Types	Recommended Input Amount	Typical Yield (µg)	Purity Ratio (A260/A280)
Cultured Cells	K562, SNU-1, U937, HeLa, NIH3T3, Vero, B16	1x10^6 cells	7 – 20	1.9 ± 0.2
Animal Tissues	Liver, Brain, Kidney, Tail (Mouse)	25 mg	10 – 24	1.9 ± 0.2
	Spleen (Mouse)	10 mg	9 – 20	1.9 ± 0.2
	Heart, Lung, Stomach, Muscle (Mouse)	25 mg	3 – 16	1.9 ± 0.2
	Hair (Human, with roots)	10 ea	10 – 20	1.9 ± 0.2
Whole Blood & Fractions	Whole Blood (EDTA / Heparin / Citrate)	200 µl	4 – 10	1.9 ± 0.2
	Buffy Coat	from 300 µl blood	6 – 12	1.9 ± 0.2
Bacterial Cultures	E. coli, P. aeruginosa, S. gallinarum	3 OD600	7 – 16	1.9 ± 0.2
Biological Swabs	Blood Swab, Buccal Swab	1 ea	2 – 15	1.9 ± 0.2
Fixed Tissues*	FFPE / Formalin-Fixed Liver (Mouse)	25 mg	< 1	ND

* Note : FFPE and formalin-fixed samples yield degraded/fragmented DNA due to cross-linking; suitable primarily for short-target PCR applications.

10. REGULATORY & WARRANTY

10.1. Quality Control Compliance (ISO Standard Verification)

- ISO Quality Management: This product is manufactured under strict quality inspection and lot-by-lot QC standards in accordance with ISO 9001 and ISO 13485 certified Quality Management Systems.
- Certificate of Analysis (CoA): All released products meet the physical, chemical, and biological activity criteria specified in the product specification, with quality compliance verified via lot-specific CoA.

10.2. Product Warranty & Limitations Statement

- Warranty Scope: The manufacturer guarantees that this product will perform as specified through its expiration date when stored and operated under recommended storage conditions.
- This warranty is strictly limited to the replacement of defective products or the refund of the purchase price.
- The manufacturer assumes no liability for failures resulting from improper storage, misuse, operator error, or unauthorized application.
- For research use only (RUO) or OEM products, final validation, regulatory clearance, and application responsibilities lie entirely with the purchaser.

11. ORDERING INFORMATION

Product Name	Cat. No.
G-spin™ for Bacteria Genomic DNA Extraction Kit	17271
i-genomic BYF DNA Extraction Mini Kit	17231
easy-spin™ Total RNA Extraction Kit	17221
Maxime PCR PreMix (i-Taq)	25025 / 25026
Maxime PCR PreMIx (i-StarTaq)	25165 / 25167
2X PCR Master mix Solution (i-StarTaq)	25166
SiZer™-1,000 DNA Marker Solution	24074
RealMOD™ Probe R^2 2X qPCR mix (with UDG)	25363.200 (200 rxns) 25363.500 (500 rxns) 25363.1000 (1000 rxns)
RealMOD™ Probe R^2 2X qRT-PCR mix (with UDG)	25364.200 (200 rxns) 25364.500 (500 rxns)

Visual Protocol (Cell gDNA Extraction)