Genemed Synthesis, Inc.
6203 Wood Lake Center Dr., Bldg. 2, San Antonio, TX 78244, USA
Toll free (800) 344-5337; **Phone**: (210) 745-5988; **Fax** (210) 745-5992
Email: info@genemedsyn.com, **Website**: www.genemedsyn.com

Product Data Sheet

Cat # V-130-50	Blood & Tissue Genomic DNA Extraction	**Size:** 50 Preps
Cat # V-130-250	Blood & Tissue Genomic DNA Extraction	**Size:** 250 Preps

Blood & Tissue Genomic DNA Extraction System

GSI Blood & Tissue Genomic DNA Extraction System provides a simple and fast way to purify genomic DNA (including viral or mitochondrial DNA) from various sources such as blood, plasma, serum, buffy coat, lymphocytes and body fluids. A simple spin column procedure can purify pure DNA (approximately 20-30 kb fragment) for PCR, enzymatic reactions, and other downstream applications. 0.2 ml whole blood volume will yield 10 µg of genomic DNA.

Downstream Application
* Restriction digestion
* Southern Blotting
* RAPD, RFLP
* PCR, Real-Time PCR

Product Contents

Cat. No	**GG0000**
Preps	4
LYS Buffer	1.5ml
EX Buffer	1.5ml
WS Buffer	1.5ml
Proteinase K	1mg
B/T Genomic DNA Mini Column	4
Collection Tube	8
Protocol	1

Shipping & Storage

The sample of B/T Genomic DNA Extraction System is shipping and storage at ambient temperature up to 6 months.

If precipitate form by freezing temperature on any buffer, warm up at 37℃ to redissolve.

Protocol

❖ **Please read the following notes before starting the procedures.**

Important Notes

- RNA may be copurified with genomic DNA. RNA will not affect PCR, but may affect certain downstream applications. If RNA-free genomic DNA is desired, add RNase A to the sample as indicated in the protocol.
- DNA can be eluted in 10 mM Tris-HCl (pH 9.0), Milli-Q, double-distilled H_2O, or TE buffer (pH 8.0). Since genomic DNA elution takes place most effectively at pH 9, to ensure optimal elution, make sure that pH of these elution solution are between 8.0 and 9.0.
- Add 6 ml of 98-100% ethanol into WS Buffer bottle when first open.
- Add 0.1 ml sterile ddH_2O to reconstitute the provided Proteinase K by vortexing. Store the solution at 4 ☐.
- Buffers in this system contain irritants. Appropriate safety apparels such as gloves and the lab coat should be worn to protect from skin contact.
- All procedure should be done at room temperature (20-25 ☐).
- Centrifuge steps done at full speed refers to 10,000 x g or 13,000-14,000 rpm of a microcentrifuge.

I. Blood Protocol:

For samples including whole blood, serum, plasma, buffy coat, body fluid, 10^6-10^7 lymphocytes and culture cells in 200 µl PBS.

1. **Pipet up to 200 µl sample into a 1.5-ml sterile eppendorf tube. When the sample volume is less than 200 µl, add PBS to make up to 200 µl.**
   If RNA-free genomic DNA is desired, add 10 µl of 50 mg/ml RNase A to the sample at this step.

2. **Add 20 µl Proteinase K and 200 µl EX Buffer into the sample. Mix immediately by vortexing for 20 seconds.**
   Do not add Proteinase K directly to EX Buffer. If sample volume is larger than 200 µl, increase the amount of EX Buffer and Proteinase K proportionally.

3. **Incubate at 60 ☐ for 20 minutes to lyse the sample. Vortex or invert the sample every 3-5 minutes during incubation.**

Genemed Synthesis, Inc.
6203 Wood Lake Center Dr., Bldg. 2, San Antonio, TX 78244, USA
Toll free (800) 344-5337; **Phone**: (210) 745-5988; **Fax** (210) 745-5992
Email: info@genemedsyn.com, **Website**: www.genemedsyn.com

Ensure complete sample lysis: whole blood sample should NOT appear viscous; buffy coat should NOT contain insoluble residues; cell sample should appear translucent.

4. **Adjust the incubator to 70 ☐ to incubate for 20 minutes.**
   Alternatively, place the sample to another 70°C incubator and incubate for 10 minutes.

5. **Preheat 10 mM Tris-HCl (pH 9.0), ddH_2O, or TE buffer (provided by user) at 70 ☐ (500 µl/prep) for DNA elution.**

6. **Add 210 µl of ethanol (98-100%) or isopropanol to the 70°C-incubated sample of Step 4 and mix by vortexing.**
   If the sample volume is larger than 200 µl, increase the amount of isopropanol or ethanol proportionally.

7. **Place a B/T Genomic DNA Mini Column onto a Collection Tube. Pipet all the mixture (including any precipitate) into the column without touching the rim.**

8. **Centrifuge at 8,000 rpm (6,000 x g) for 2 minutes. Place the column onto a new Collection Tube.**

9. **Wash the column twice with 0.5 ml WS Buffer by centrifuging at 8,000 rpm (6,000 x g) for 2 minutes. Discard the flow-through.**
   Add 6 ml of ethanol (98-100%) when first open the WS Buffer bottle.

10. **Centrifuge the column at full speed for another 2 minutes to remove ethanol residue.**

11. **Place the column onto a new 1.5-ml tube (provided by user). Elute DNA with 200 µl of the preheated elution solution from Step 5.**

12. **Stand the column for 1-5 minutes, and centrifuge for 1-2 minutes to elute DNA.**

13. **Store eluted DNA at 4 ☐ or –20 ☐.**
    Store DNA at 4 ☐ for frequent use or at –20 ☐ for long-term storage. Repeated freeze-thaw cycles can cause shearing of genomic DNA.

II. Tissue Protocol:

1. **Cut 30 mg of tissue (15 mg spleen) into small pieces and place the sample into a 1.5-ml sterile eppendorf tube. Add 200 µl LYS Buffer and homogenize the sample.**
   If the sample size is larger than 30 mg, increase the amount of LYS Buffer proportionally.

2. **Add 20 µl Proteinase K to the sample. Mix immediately by vortexing for 20 seconds.**
   If RNA-free genomic DNA is desired, add 10 µl of 50 mg/ml RNase A to the sample.

3. **Incubate at 60 ☐ for 1 hour to lyse the sample. If tissue is difficult to lyse, increase the incubation time to 2-3 hours. Vortex or invert the sample every 10-15 minutes.**
   Ensure complete sample lysis; sample after complete lysis should appear translucent.

4. **Adjust the incubator to 70 ☐ to incubate for 20 minutes.**
   Alternatively, place the sample to another 70 ☐ incubator and incubate for 10 minutes.

5. **Meanwhile, preheat 10 mM Tris-HCl (pH 9.0), ddH_2O, or TE buffer (provided by user) at 70 ☐ (500 µl/prep) for DNA elution.**

6. **Add 200 µl of EX Buffer to the sample, mix by vortexing and incubate at 70 ☐ for 10 minutes.**

7. **Add 210 µl of ethanol (98-100%) or isopropanol to the sample and mix by vortexing.**
   If the sample mixture is more than 400 µl, increase the amount of ethanol proportionally.

8. **Place a B/T Genomic DNA Mini Column onto a Collection Tube. Pipet all the mixture (including any precipitate) into the column without touching the rim. Centrifuge at 8,000 rpm (6,000 x g) for 2 minutes. Place the column onto a new Collection Tube.**
   If a precipitate formed from step 7, apply the precipitate and mixture to the B/T Genomic DNA Mini Column.
   If the B/T Genomic DNA Mini Column clogged after 2 minutes spin, centrifuge again at full speed for another 2 minutes.

Genemed Synthesis, Inc.
6203 Wood Lake Center Dr., Bldg. 2, San Antonio, TX 78244, USA
Toll free (800) 344-5337; **Phone**: (210) 745-5988; **Fax** (210) 745-5992
Email: info@genemedsyn.com, **Website**: www.genemedsyn.com

9. Wash the column twice with 0.5 ml WS Buffer by centrifuging at 8,000 rpm (6,000 x g) for 2 minutes. Discard the flow-through.

Ensure that ethanol has been added into WS Buffer bottle when first open.

10. Centrifuge the column at full speed for another 2 minutes to remove ethanol residue.

11. Place the column onto a new 1.5-ml tube (provided by user). Elute DNA with 200 µl of the preheated elution solution from Step 5.

12. Stand the column for 1-5 minutes, and centrifuge for 1-2 minutes to elute DNA.

13. Store eluted DNA at 4 ☐ or –20 ☐.

III. Mouse Tail protocol:

1. **Cut into small pieces of a segment of mouse tail of up to 0.5 cm. Place the sample into a 1.5-ml sterile tube.**
   Segment close to the tail tip is preferred. Segment away from the tip is thicker and takes longer time to lyse completely.

2. **Add 20 µl Proteinase K and 200 µl LYS Buffer to the sample. Mix immediately by vortexing for 20 seconds.**
   If RNA-free genomic DNA is desired, add 10 µl of 50 mg/ml RNase A to the sample.
   Further addition of 20 µl of 10 mg/ml Proteinase E (DNase-free, provided by user) can enhance mouse tail lysis and increase DNA yield.

3. **Incubate at 60 ☐ for 1-4 hours or overnight to lyse the tail tissue. Vortex or invert the sample every 20-30 minutes during incubation.**
   Ensure complete sample lysis; sample after complete lysis should appear translucent with only hair and bone residues remained.

4. **Follow the Tissue Protocol starting from Step 4 on Page 2.**

IV. Paraffin-Embedded Tissue Protocol

1. **Cut a small section of paraffin-embedded tissue (about 25 mg) and put into a 1.5-ml eppendorf tube.**

2. **Add 1 ml xylene and incubate at room temperature with occasional mixing for 30 minutes to extract paraffin from tissue.**

3. **Centrifuge at full speed for 5 minutes. Remove the supernatant by pipetting.**

4. **Add 1 ml absolute ethanol to the tissue pellet, mix, and centrifuge at full speed for 5 minutes. Remove ethanol-containing xylene residue by pipetting.**

5. **Evaporate ethanol residue by incubating at 37 ☐ for 10 minutes.**

6. **Resuspend the pellet in 200 µl LYS Buffer.**

7. **Follow the Tissue Protocol starting from Step 2 on Page 2.**

V. Bacteria Protocol

A. For Gram-positive and Gram-negative bacteria

1. **Pellet log-phase grown bacteria of up to 10^9 (or up to 3 ml culture) at 7,500 rpm (5,000 x g) for 10 minutes.**

2. **Resuspend the pellet in 200 µl lysozyme reaction solution (20 mM Tris-HCl, pH 8.0; 2 mM EDTA; 20 mg/ml lysozyme).**
   If RNA-free genomic DNA is desired, add 10 µl of 50 mg/ml RNase A to the resuspended cells.

3. **Incubate at 37 ☐ for 30 minutes.**

4. **Add 20 µl Proteinase K and 200 µl EX Buffer to the sample. Mix immediately by vortexing for 20 seconds.**

5. **Incubate at 60 ☐ for 30 minutes and then at 70 ☐ for a further 30 minutes to lyse the bacterial cells. Vortex or invert the sample every 5 minutes during incubation.**
   Incubation with mixing facilitates lysis. Ensure complete cell lysis; sample after complete lysis should appear translucent.

Genemed Synthesis, Inc.
6203 Wood Lake Center Dr., Bldg. 2, San Antonio, TX 78244, USA
Toll free (800) 344-5337; **Phone**: (210) 745-5988; **Fax** (210) 745-5992
Email: info@genemedsyn.com, **Website**: www.genemedsyn.com

6. Meanwhile, preheat 10 mM Tris-HCl (pH 9.0), ddH_2O, or TE buffer (provided by user) at 70 □ (500 µl/prep) for DNA elution.

7. Follow the Tissue Protocol starting from Step 7 on Page 3.

B. For bacteria in biological fluids

1. Pellet cells by centrifuging at 7,500 rpm (5,000 x g) for 10 minutes.

2. Resuspend the pellet in 200 µl LYS Buffer.
If RNA-free genomic DNA is desired, add 5 µl of 50 mg/ml RNase A to the resuspended cells.

3. Follow the Tissue Protocol starting from Step 2 on Page 2.

C. For bacteria from eye, nasal, or pharygeal swabs

1. Collect bacterial cells by rinsing and soaking the swabs in 2 ml PBS at room temperature for 2-3 hours.

2. Pellet cells by centrifuging at 7,500 rpm (5,000 x g) for 10 minutes.

3. Resuspend the pellet in 200 µl LYS Buffer.
If RNA-free genomic DNA is desired, add 10 µl of 50 mg/ml RNase A to the resuspended cells.

4. Follow the Tissue Protocol starting from Step 2 on Page 2.

VI. Yeast Protocol

1. Pellet log-phase grown yeast cells up to 10^8 (or up to 3 ml culture) at 7,500 rpm (5,000 x g) for 10 minutes.

2. Resuspend the pellet in 500 ml sorbitol reaction solution (1 M sorbitol; 100 mM EDTA; 14 mM β-mercaptoethanol; 200 U lyticase or zymolase).
If RNA-free genomic DNA is desired, add 10 µl of 50 mg/ml RNase A to the resuspended cells.

3. Incubate at 30□ for 30 minutes.

4. Centrifuge at 7,500 rpm (5,000 x g) for 5 minutes. Resuspend the pellet in 200 µl LYS Buffer.

5. Follow the Tissue Protocol starting from Step 2 on Page 2.

VII. Virus Protocol

1. Prepare viral DNA from blood or body fluid, the **Blood Protocol** is recommended.

2. Prepare integrated viral DNA, the **Tissue Protocol** is recommended.

Troubleshooting

1. Brown color residues remain on the membrane of Genomic DNA column after washing

a. Incomplete digestion of Hemoglobin
Vortex the sample after Proteinase K is added. Mix the sample every 3-5 minutes during incubation.

b. No alcohol added to the sample before loading onto the Genomic DNA column
Before passing the column, add 210 µl (or suitable volume) of absolute ethanol or isopropanol to the sample.

c. Incorrect amount of ethanol added to the WS Buffer
Make sure that ethanol is added into the WS Buffer bottle when first open.

2. Little or no DNA in the elute

a. Sample contains too low amount of genomic DNA
Increase the sample amount, Proteinase K, and buffer proportionally. If the sample is whole blood, prepare buffy coat from a larger volume of blood.

b. Blood sample is not lysed completely
Add another 20 µl fresh Proteinase K per sample and repeat incubation.

c. No alcohol added to the sample before loading onto the Genomic DNA column
Before passing the column, add 210 µl (or suitable volume) of absolute ethanol or isopropanol to the sample.

d. Incorrect amount of ethanol added to the WS Buffer

Genemed Synthesis, Inc.
6203 Wood Lake Center Dr., Bldg. 2, San Antonio, TX 78244, USA
Toll free (800) 344-5337; **Phone**: (210) 745-5988; **Fax** (210) 745-5992
Email: info@genemedsyn.com, **Website**: www.genemedsyn.com

Make sure that ethanol is added into the WS Buffer bottle when first open.

e. Elution solution is not preheated at 70 ☐

Preheat the elution solution at 70 ☐ before used.

f. The pH value of the elution solution is too low

Make sure that the pH value of 10 mM Tris-HCl, ddH_2O or TE buffer for elution is between 8.0-9.0.

3. Column is clogged when passing the sample

a. Tissue sample contains undigested remains

After Proteinase K digestion, centrifuge the sample at full speed for 5 minutes to remove undigested remains.

b. Blood sample contains clots

Use whole blood sample mixed well with anti-coagulant to prevent formation of blood clot.

Do not use blood clot for genomic DNA extraction.

c. Sample is very viscous

Too much sample is used. Reduce the sample amount.

4. A_{260}/A_{280} ratio of eluted genomic DNA is low

a. Protein in the sample is not completely degraded

Vortex the sample after Proteinase K is added. Mix the sample at constant intervals during incubation. Add 20 µl fresh Proteinase K per sample and continue incubation.

b. No alcohol added to the sample before loading onto the Genomic DNA column

Before passing the column, add 210 µl (or suitable volume) of absolute ethanol or isopropanol to the sample.

c. Eluted genomic DNA contains contaminants.

Do not touch the rim of the column during sample or buffer loading.

d. Eluted genomic DNA contains ethanol

After the final wash, centrifuge the column at full speed for another 2 minutes to remove the ethanol residue completely.

e. Using ddH_2O of acidic pH (5.0-6.0) to dilute DNA samples for spectrophotometric analysis

Use 10 mM Tris-HCl of pH 7.5 or TE buffer to dilute the DNA samples.

5. A_{260}/A_{280} ratio for genomic DNA is high (over 1.9)

a. RNA contamination

Add RNase A to the sample as described in the protocol.

6. Genomic DNA appears smearing and degraded

a. Sample is not fresh or stored improperly for a long time

Flash freeze fresh samples in liquid nitrogen and store at –80 ☐ if not used immediately.

b. Blood sample is not fresh or stored improperly for a long time

Use fresh blood, or blood stored at room temperature for fewer than 2 days.

c. Gel electrophoresis is performed in used running buffer contaminated with DNase

Use fresh TAE or TBE running buffer for electrophoresis.

d. Paraffin-embedded tissue is used as sample

Genomic DNA isolated from this kind of sample is usually degraded. It is still suitable for PCR application, but is not recommended for Southern blotting and restriction analysis

All buffers need to be mixed well before use.