**For research use only**

Catalogue Number
GS004, GS100, GS300

**Instruction Manual Download**
When using this product for the first time, or if you are unfamiliar with the procedure, please scan the QR code and download the complete instruction manual.

**IMPORTANT BEFORE USE!**

1. Add absolute ethanol (see the bottle label for volume) to Wash Buffer then mix by shaking for a few seconds. Check the box on the bottle. Be sure and close the bottle tightly after each use to avoid ethanol evaporation.

2. Add ddH2O pH 7.0-8.5 (see the bottle label for volume) to Proteinase K then vortex to ensure it is completely dissolved. Check the box on the bottle. Once it is dissolved completely, centrifuge for a few seconds to spin down the mixture. For extended periods, the ddH2O and Proteinase K mixture should be stored at 4°C. Use only fresh ddH2O as ambient CO₂ can quickly cause acidification.

3. Prepare Phosphate Buffered Saline (PBS, pH 7.2) for blood, serum, plasma, cultured cells and FFPE tissue samples.

4. Prepare Xylene for FFPE tissue samples.

5. Yield and quality of DNA will be higher when fresh samples or samples which have been flash frozen and stored at -20°C or -70°C are used. DNA in FFPE or tissue which has been repeatedly frozen and thawed may be degraded.

6. Optionally prepare RNase A (50 mg/ml) for RNA-free DNA when performing sensitive downstream reactions. However, residual RNA will not affect PCR.

**Tissue, FFPE, Insect and Sperm Protocol Procedure**

**1A. Tissue Sample Dissociation**
Transfer up to 25 mg of fresh animal tissue (0.5 cm mouse tail x 2 or 0.5 cm rat tail x 1) to a 1.5 ml microcentrifuge tube. If the tissue has a higher number of cells (e.g. spleen or liver), reduce the starting material to 10 mg. Add 200 μl of GST Buffer and 20 μl of Proteinase K then vortex thoroughly. Incubate at 60°C overnight or until the sample lysate becomes clear. During incubation, transfer the required volume of Elution Buffer (200 μl/sample) to a 1.5 ml microcentrifuge tube and heat to 60°C (for Step 5 DNA Elution).

**1B. FFPE Sample Preparation**
Cut up to 25 mg sections of FFPE and transfer to a 1.5 ml microcentrifuge tube. Using a sterile blade is recommended. Add 1 ml of xylene then mix by shaking vigorously. Incubate at room temperature for approximately 10 minutes (shake occasionally during incubation). Centrifuge at 14-16,000 x g for 3 minutes then remove the supernatant. Add 1 ml of absolute ethanol to wash the sample pellet and mix by inverting. Centrifuge at 14-16,000 x g for 3 minutes then remove the supernatant. Open the tube and incubate at 37°C for 15 minutes to evaporate ethanol residue. Add 200 μl of GST Buffer and 20 μl of Proteinase K then vortex thoroughly. Incubate at 60°C overnight or until the sample lysate becomes clear. During incubation, transfer the required volume of Elution Buffer (200 μl/sample) to a 1.5 ml microcentrifuge tube and heat to 60°C (for Step 5 DNA Elution).

**1C. Insect Sample Preparation**
Transfer up to 50 mg of insect tissue to a mortar. Add liquid nitrogen to the mortar and grind the tissue thoroughly using a pestle. Refill the mortar occasionally with liquid nitrogen to keep the sample frozen. Transfer the tissue powder to a 1.5 ml microcentrifuge tube. Add 200 μl of GST Buffer and 20 μl of Proteinase K then vortex thoroughly. Incubate at 60°C for 1-3 hours or until the sample lysate becomes clear. During incubation, invert the tube occasionally and transfer the required volume of Elution Buffer (200 μl/sample) to a 1.5 ml microcentrifuge tube and heat to 60°C (for Step 5 DNA Elution).

**1D. Sperm Sample Preparation**
Add RNase-free water to DTT powder (see the bottle label for volume) then vortex to dissolve. Spin down the solution. The solution should be stored at -20°C. Transfer 900 μl of Sperm Lysis Buffer into a 1.5 ml microcentrifuge tube. Add 80 μl of DTT solution and 20 μl of Proteinase K immediately before use. Mix well by vortex.

NOTE: Sperm Lysis Buffer and DTT can be purchased directly from Geneaid.

Add 100 μl of sperm and 100 μl of fresh prepared Sperm Lysis Buffer (containing DTT and proteinase K) into a new 1.5 ml microcentrifuge tube, mix by vortex then incubate at 60°C for 1 hour to dissolve the sample. During incubation, invert the tube occasionally and transfer the required volume of Elution Buffer (200 μl/sample) to a 1.5 ml microcentrifuge tube and heat to 60°C (for Step 5 DNA Elution).
2. Cell Lysis
If insoluble material remains following incubation, centrifuge for 2 minutes at 14-16,000 x g then carefully transfer the supernatant to a new 1.5 ml microcentrifuge tube. Add **200 μl of GSB Buffer** and shake vigorously for 10 seconds.

NOTE: It is essential that the sample and GSB Buffer are mixed thoroughly to yield a homogeneous solution.

Optional RNA Removal Step
For RNA-free gDNA, following GSB Buffer addition, add 5 μl of RNase A (50 mg/ml) and mix by shaking vigorously. Incubate at room temperature for 5 minutes to ensure efficient RNA degradation.

3. DNA Binding
Add **200 μl of absolute ethanol** to the sample lysate and mix **IMMEDIATELY** by shaking vigorously for 10 seconds. If precipitate appears, break it up as much as possible with a pipette. Place a **GS Column in a 2 ml Collection Tube**. Transfer all of the mixture (including any insoluble precipitate) to the GS Column. Centrifuge at 14-16,000 x g for 1 minute. Following centrifugation, if the mixture did not flow through the GS Column membrane, increase the centrifuge time until it passes completely. Discard the 2 ml Collection Tube containing the flow-through then transfer the GS Column to a new 2 ml Collection Tube.

NOTE: It is important that the lysate and ethanol are mixed thoroughly to yield a homogeneous solution.

4. DNA Elution
Transfer the dried GS Column to a clean 1.5 ml microcentrifuge tube. Add **100 μl of pre-heated Elution Buffer**, TE Buffer or water into the **CENTER** of the column matrix. Let stand for at least 3 minutes to allow Elution Buffer, TE Buffer or water to be completely absorbed. Centrifuge at 14-16,000 x g for 30 seconds to elute purified DNA.

5. Optional RNA Removal Step
For RNA-free gDNA, following GSB Buffer addition and 60ºC incubation, add 5 μl of RNase A (50 mg/ml) and mix by shaking vigorously. Incubate at room temperature for 5 minutes to ensure efficient RNA degradation.

Following incubation, centrifuge for 5 minutes at 3,000 x g then transfer the supernatant to a new 1.5 ml microcentrifuge tube.

2. DNA Binding
Add **200 μl of absolute ethanol** to the sample lysate and mix **IMMEDIATELY** by shaking vigorously for 10 seconds. If precipitate appears, break it up as much as possible with a pipette. Place a **GS Column in a 2 ml Collection Tube**. Transfer all of the mixture (including any insoluble precipitate) to the GS Column. Centrifuge at 14-16,000 x g for 1 minute. Following centrifugation, if the mixture did not flow through the GS Column membrane, increase the centrifuge time until it passes completely. Discard the 2 ml Collection Tube containing the flow-through then transfer the GS Column to a new 2 ml Collection Tube.

NOTE: It is important that the lysate and ethanol are mixed thoroughly to yield a homogeneous solution.

Hair Protocol Procedure

1. Cell Lysis
Cut off a **0.5-1 cm piece from at least 10 hair bulbs, including follicle cells** and transfer to a 1.5 ml microcentrifuge tube. Add **200 μl of GST Buffer and 20 μl of Proteinase K (making sure the hair is completely submerged)** and mix by shaking. Incubate at 60ºC for 30 minutes to lyse the sample. During incubation, shake the tube every 5 minutes and transfer the required volume of Elution Buffer (200 μl/sample) to a 1.5 ml microcentrifuge tube and heat to 60ºC (for Step 4 DNA Elution). Add **200 μl of GSB Buffer** and mix vigorously. Incubate at 60ºC for 20 minutes. During incubation, shake the tube every 5 minutes.

Optional RNA Removal Step
For RNA-free gDNA, following GSB Buffer addition and 60ºC incubation, add 5 μl of RNase A (50 mg/ml) and mix by shaking vigorously. Incubate at room temperature for 5 minutes to ensure efficient RNA degradation.

Following incubation, centrifuge for 5 minutes at 3,000 x g then transfer the supernatant to a new 1.5 ml microcentrifuge tube.
3. Wash
Add 400 μl of W1 Buffer to the GS Column. Centrifuge at 14-16,000 x g for 30 seconds then discard the flow-through. Place the GS Column back in the 2 ml Collection Tube. Add 600 μl of Wash Buffer (make sure absolute ethanol was added) to the GS Column. Centrifuge at 14-16,000 x g for 30 seconds then discard the flow-through. Place the GS Column back in the 2 ml Collection Tube. Centrifuge again for 3 minutes at 14-16,000 x g to dry the column matrix.

4. Elution
Transfer the dried GS Column to a clean 1.5 ml microcentrifuge tube. Add 100 μl of pre-heated Elution Buffer, TE Buffer or water into the CENTER of the column matrix. Let stand for at least 3 minutes to allow Elution Buffer, TE Buffer or water to be completely absorbed. Centrifuge at 14-16,000 x g for 30 seconds to elute purified DNA.

Blood, Cultured Cell, Amniotic Fluid Protocol Procedure

1A. Blood Sample Preparation
Transfer up to 200 μl of whole blood, serum, plasma, buffy coat or body fluids to a 1.5 ml microcentrifuge tube. Adjust the volume to 200 μl with PBS. Add 20 μl of Proteinase K then mix by pipetting. Incubate at 60ºC for 5 minutes.

1B. Cultured Cell Sample Preparation
Trypsinze adherent cells prior to harvesting. Transfer cells (up to 1 x 10^7) to a 1.5 ml microcentrifuge tube then centrifuge for 5 minutes at 300 x g. Discard the supernatant then resuspend cells in 200 μl of PBS by pipette. Add 20 μl of Proteinase K then mix by pipetting. Incubate at 60ºC for 5 minutes.

1C. Amniotic Fluid Sample Preparation
Transfer up to 15 ml of amniotic fluid to a 15 ml centrifuge tube. Centrifuge for 3 minutes at 14-16,000 x g then discard the supernatant. Add 200 μl of GST Buffer to resuspend the pellet and transfer the mixture to a 1.5 ml microcentrifuge tube. Add 10 μl of Proteinase K and shake vigorously. Incubate at 60ºC for 30 minutes. During incubation, invert the tube every 5 minutes.

2. Cell Lysis
Add 200 μl of GSB Buffer then mix by shaking vigorously. For blood and cell samples, incubate at 60ºC for 5 minutes, inverting the tube every 2 minutes. For amniotic fluid samples, incubate at 60ºC for at least 20 minutes, inverting the tube every 5 minutes. During incubation, transfer required volume of Elution Buffer (200μl/sample) to a 1.5 ml microcentrifuge tube and heat to 60ºC (for Step 5 DNA Elution).

Optional RNA Removal Step
For RNA-free gDNA, following GSB Buffer addition and 60ºC incubation, add 5 μl of RNase A (50 mg/ml) and mix by shaking vigorously. Incubate at room temperature for 5 minutes to ensure efficient RNA degradation.

3. DNA Binding
Add 200 μl of absolute ethanol to the sample lysate and mix IMMEDIATELY by shaking vigorously for 10 seconds. If precipitate appears, break it up as much as possible with a pipette. Place a GS Column in a 2 ml Collection Tube. Transfer all of the mixture (including any insoluble precipitate) to the GS Column. Centrifuge at 14-16,000 x g for 1 minute. Following centrifugation, if the mixture did not flow through the GS Column membrane, increase the centrifuge time until it passes completely. Discard the 2 ml Collection Tube containing the flow-through then transfer the GS Column to a new 2 ml Collection Tube. 

NOTE: It is important that the lysate and ethanol are mixed thoroughly to yield a homogeneous solution.

4. Wash
Add 400 μl of W1 Buffer to the GS Column. Centrifuge at 14-16,000 x g for 30 seconds then discard the flow-through. Place the GS Column back in the 2 ml Collection Tube. Add 600 μl of Wash Buffer (make sure absolute ethanol was added) to the GS Column. Centrifuge at 14-16,000 x g for 30 seconds then discard the flow-through. Place the GS Column back in the 2 ml Collection Tube. Centrifuge again for 3 minutes at 14-16,000 x g to dry the column matrix.

5. Elution
Transfer the dried GS Column to a clean 1.5 ml microcentrifuge tube. Add 100 μl of pre-heated Elution Buffer, TE Buffer or water into the CENTER of the column matrix. Let stand for at least 3 minutes to allow Elution Buffer, TE Buffer or water to be completely absorbed. Centrifuge at 14-16,000 x g for 30 seconds to elute purified DNA.
### Kit Components

<table>
<thead>
<tr>
<th>Component</th>
<th>GS004</th>
<th>GS100</th>
<th>GS300</th>
</tr>
</thead>
<tbody>
<tr>
<td>GST Buffer</td>
<td>3 ml</td>
<td>30 ml</td>
<td>75 ml</td>
</tr>
<tr>
<td>GSB Buffer</td>
<td>4 ml</td>
<td>40 ml</td>
<td>75 ml</td>
</tr>
<tr>
<td>W1 Buffer</td>
<td>2 ml</td>
<td>45 ml</td>
<td>130 ml</td>
</tr>
<tr>
<td>Wash Buffer(^1) (Add Ethanol)</td>
<td>1 ml (4 ml)</td>
<td>25 ml (100 ml)</td>
<td>50 ml (200 ml)</td>
</tr>
<tr>
<td>Proteinase K(^2) (Add ddH(_2)O)</td>
<td>1 mg (0.10 ml)</td>
<td>11 mg x 2 (1.10 ml)</td>
<td>65 mg (6.50 ml)</td>
</tr>
<tr>
<td>Elution Buffer</td>
<td>1 ml</td>
<td>30 ml</td>
<td>75 ml</td>
</tr>
<tr>
<td>GS Columns</td>
<td>4</td>
<td>100</td>
<td>300</td>
</tr>
<tr>
<td>2 ml Collection Tubes</td>
<td>8</td>
<td>200</td>
<td>600</td>
</tr>
</tbody>
</table>

1. Add absolute ethanol (see the bottle label for volume) to Wash Buffer then mix by shaking for a few seconds. Check the box on the bottle. Be sure and close the bottle tightly after each use to avoid ethanol evaporation.

2. Add ddH\(_2\)O pH7.0-8.5 (see the bottle label for volume) to Proteinase K then vortex to ensure Proteinase K is completely dissolved. Check the box on the bottle. Once it is dissolved completely, centrifuge for a few seconds to spin down the mixture. For extended periods, the ddH\(_2\)O and Proteinase K mixture should be stored at 4\(^\circ\)C. Use only fresh ddH\(_2\)O as ambient CO\(_2\) can quickly cause acidification.

### The gSYNC™ DNA Extraction Kit Functional Test Data

**Figure 1.** Genomic DNA from 50, 100 and 200 µl whole blood samples was extracted using the gSYNC™ DNA Extraction Kit. 10 µl from 100 µl eluates of purified genomic DNA was analyzed by electrophoresis on a 0.8% agarose gel.

<table>
<thead>
<tr>
<th>Volume</th>
<th>Yield</th>
<th>260/280</th>
</tr>
</thead>
<tbody>
<tr>
<td>50 µl</td>
<td>1.54 µg</td>
<td>1.85</td>
</tr>
<tr>
<td>100 µl</td>
<td>2.70 µg</td>
<td>1.87</td>
</tr>
<tr>
<td>200 µl</td>
<td>5.56 µg</td>
<td>1.90</td>
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</table>

**Storage:** dry at room temperature (15-25\(^\circ\)C)