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For Research Use Only


IBI SCIENTIFIC

# INSTRUCTION MANUAL

## gMAX DNA Mini Kit

IB47280 (4 Preparation Sample Kit)

IB47281 (100 Preparation Kit)

IB47282 (300 Preparation Kit)

# Advantages

**Sample**: tissue, rodent tails, ear punches, fresh or frozen blood, serum, plasma, buy coat, body uids, cultured cells, amniotic uid, FFPE, hair, insects, sperm

**Yield**: up to 6 µg of gDNA from 200 µl of fresh whole blood samples

**Format**: genomic DNA spin column

**Operation Time**: within 20 minutes

**Elution Volume**: 30-100 µl

**Kit Storage**: dry at room temperature (15-25°C)

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# Introduction

The gMAX DNA Mini Kit is optimized for genomic, mitochondrial and virus DNA purification from whole blood (fresh blood and frozen blood), tissue, formalin-fixed paraffin-embedded tissue (FFPE), amniotic fluid, insects and sperm in one convenient kit. This DNA extraction kit uses Proteinase K and chaotropic salt to lyse cells and degrade protein, allowing DNA to bind to the glass fiber matrix of the spin column. Contaminants are removed using a Wash Buffer and the purified genomic DNA is eluted by a low salt Elution Buffer, TE or water. The entire procedure can be completed within 20 minutes without phenol/chloroform extraction or alcohol precipitation. The purified DNA (approximately 20-30 kb) is suitable for use in PCR or other enzymatic reactions.

# Quality Control

The quality of the gMAX DNA Mini Kit is tested on a lot-to-lot basis by isolating genomic DNA from 200 µl of whole human blood. The purified DNA (5 µg with an A260/A280 ratio of 1.8-2.0) is quantified with a spectrophotometer and analyzed by electrophoresis.

# Kit Components

| Component | IB47280 | IB47281 | IB47282 |
|---|---|---|---|
| GST Buffer | 3 ml | 30 ml | 75 ml |
| GSB Buffer | 4 ml | 40 ml | 75 ml |
| W1 Buffer | 2 ml | 45 ml | 130 ml |
| Wash Buffer[1] (Add Ethanol) | 1 ml (4 ml) | 25 ml (100 ml) | 50 ml (200 ml) |
| Proteinase K[2] (Add ddH2O) | 1 mg (0.10 ml) | 11 mg x 2 (1.10 ml) | 65 mg (6.50 ml) |
| Elution Buffer | 1 ml | 30 ml | 75 ml |
| GS Columns | 4 | 100 | 300 |
| 2 ml Collection Tubes | 12 | 200 | 600 |

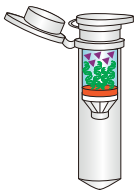
[1] Add absolute ethanol (see the bottle label for volume) to Wash Buffer then mix by shaking for a few seconds. Check the box on the bottle. Be sure and close the bottle tightly after each use to avoid ethanol evaporation.

[2] Add $ddH_2O$ to Proteinase K (see the bottle label for volume) 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 the mixture down. For extended periods, the $ddH_2O$ and Proteinase K mixture should be stored at 4°C. Use only fresh $ddH_2O$ as ambient $CO_2$ can quickly cause acidification.

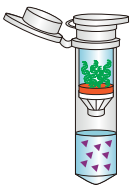
**During the procedure, always wear a lab coat, disposable gloves, and protective goggles**

# Quick Protocol Diagram

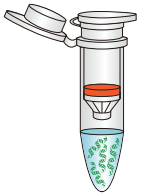
Sample preparation and cell lysis



DNA binding to membrane while contaminants remain suspended



Wash (removal of contaminants while DNA remains bound to membrane)



Elution of pure genomic DNA which is ready for subsequent reactions

# gMAX DNA Extraction Kit Protocol

Please read the entire instruction manual prior to starting the Protocol Procedure.

**IMPORTANT BEFORE USE!**

1. 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 $ddH_2O$ pH7.0-8.5 (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 $ddH_2O$ and Proteinase K mixture should bestored at 4°C. Use only fresh $ddH_2O$ as ambient CO2 can quickly cause acidification.
3. Prepare Phosphate Buffered Saline (PBS, pH7.2) 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.

Additional Requirements:
1.5 ml microcentrifuge tubes

# Solid Tissue Protocol Procedure

### *1. Tissue 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 60oC overnight or until the sample lysate becomes clear.

**NOTE!** Tissue homogenization prior to incubation will facilitate Proteinase K digestion and cell lysis subsequently increasing DNA yield. Inverting the sample occasionally during incubation will also facilitate Proteinase K digestion and cell lysis. Using an auto shaking system is more convenient when incubating samples. 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).

### *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** then 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. Wash*

Add **400 µl of W1 Buffer to the GS Column**. Centrifuge at 14-16,000 x g for 30 seconds. 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 for 3 minutes at 14-16,000 x g to dry the column matrix.

**NOTE!** Additional centrifugation at 14-16,000 x g for 5 minutes or incubation at 60oC for 5 minutes will completely dry the GS Column to avoid any residual ethanol carryover and ensure the most effective downstream applications.

### *5. Elution*

Standard elution volume is 100 µl. If less sample is to be used, reduce the elution volume (30-50 µl) to increase DNA concentration. If higher DNA yield is required, repeat the DNA Elution step to increase DNA recovery and the total elution volume to approximately 200 µl.

Transfer the dried **GS Column** to a clean 1.5 ml microcentrifuge tube. Add **100 µl of pre-heated Elution Buffer[1]** TE Buffer[2] or water[3] 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 the purified DNA.

[1]Ensure that Elution Buffer (10 mM Tris-HCl, pH8.5 at 25°C) is added into the center of the GS Column matrix and is completely absorbed.

[2]Using TE (10 mM Tris-HCl, 1 mM EDTA, pH8.0) for elution is beneficial as EDTA preserves DNA for long term storage. However, EDTA will affect PCR and other sensitive downstream applications. Ensure that TE is added into the center of the GS Column matrix and is completely absorbed.

[3]If using water for elution, ensure the water pH is between 7.0 and 8.5. dd$H_2O$ should be fresh as ambient $CO_2$ can quickly cause acidification. Ensure that water is added into the center of the GS Column matrix and is completely absorbed. DNA Eluted in water should be stored at -20°C to avoid degradation.

# Blood Protocol Procedure

### *1. 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 60°C for 5 minutes.

**NOTE!** Fresh blood is recommended. However, frozen or blood treated with anticoagulants (EDTA etc.) can also be used. Increased storage length decreases DNA yield. If using nucleated blood (e.g. bird or fish) use up to 10 µl then adjust volume to 200 µl with PBS.

### *2. Cell Lysis*

Add **200 µl of GSB Buffer** then mix by shaking vigorously. Incubate at 60°C for 5 minutes, inverting the tube every 2 minutes.

**NOTE!** It is essential that the sample and GSB Buffer are mixed thoroughly to yield a homogenous solution. Inverting the sample occasionally during incubation will facilitate Proteinase K digestion and cell lysis. Using an auto shaking system is more convenient when incubating samples. 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).

### *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 for 3 minutes at 14-16,000 x g to dry the column matrix.

**NOTE!** Additional centrifugation at 14-16,000 x g for 5 minutes or incubation at 60oC for 5 minutes will completely dry the GS Column to avoid any residual ethanol carryover and ensure the most effective downstream applications.

### *5. Elution*

Standard elution volume is 100 µl. If less sample is to be used, reduce the elution volume (30-50 µl) to increase DNA concentration. If higher DNA yield is required, repeat the DNA Elution step to increase DNA recovery and the total elution volume to approximately 200 µl.

Transfer the dried **GS Column** to a clean 1.5 ml microcentrifuge tube. Add **100 µl of pre-heated Elution Buffer[1]**, TE Buffer[2] or water[3] 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 the purified DNA.

[1]Ensure that Elution Buffer (10 mM Tris-HCl, pH8.5 at 25°C) is added into the center of the GS Column matrix and is completely absorbed.

[2]Using TE (10 mM Tris-HCl, 1 mM EDTA, pH8.0) for elution is beneficial as EDTA preserves DNA for long term storage. However, EDTA will affect PCR and other sensitive downstream applications. Ensure that TE is added into the center of the GS Column matrix and is completely absorbed.

[3]If using water for elution, ensure the water pH is between 7.0 and 8.5. dd$H_2O$ should be fresh as ambient $CO_2$ can quickly cause acidification. Ensure that water is added into the center of the GS Column matrix and is completely absorbed. DNA Eluted in water should be stored at -20°C to avoid degradation.

# Cultured Cell Protocol Procedure

### *1. 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 60°C for 5 minutes.

### *2. Cell Lysis*

Add **200 µl of GSB Buffer** then mix by shaking vigorously. Incubate at 60°C for 5 minutes, inverting the tube every 2 minutes.

**NOTE!** It is essential that the sample and GSB Buffer are mixed thoroughly to yield a homogenous solution. Inverting the sample occasionally during incubation will facilitate Proteinase K digestion and cell lysis. Using an auto shaking system is more convenient when incubating samples. 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).

### *Optional RNA Removal Step*

For RNA-free gDNA, following GSB Buffer addition and 60°C 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.

**NOTE!** Additional centrifugation at 14-16,000 x g for 5 minutes or incubation at 60°C for 5 minutes will completely dry the GS Column to avoid any residual ethanol carryover and ensure the most effective downstream applications.

### *5. Elution*

Standard elution volume is 100 µl. If less sample is to be used, reduce the elution volume (30-50 µl) to increase DNA concentration. If higher DNA yield is required, repeat the DNA Elution step to increase DNA recovery and the total elution volume to approximately 200 µl.

Transfer the dried **GS Column** to a clean 1.5 ml microcentrifuge tube. Add **100 µl of pre-heated Elution Buffer**[1], TE Buffer[2] or water[3] 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 the purified DNA.

[1]Ensure that Elution Buffer (10 mM Tris-HCl, pH8.5 at 25°C) is added into the center of the GS Column matrix and is completely absorbed.

[2]Using TE (10 mM Tris-HCl, 1 mM EDTA, pH8.0) for elution is beneficial as EDTA preserves DNA for longterm storage. However, EDTA will affect PCR and other sensitive downstream applications. Ensure that TE is added into the center of the GS Column matrix and is completely absorbed.

[3]If using water for elution, ensure the water pH is between 7.0 and 8.5. dd$H_2O$ should be fresh as ambient $CO_2$ can quickly cause acidification. Ensure that water is added into the center of the GS Column matrix and is completely absorbed. DNA Eluted in water should be stored at -20°C to avoid degradation.

# Amniotic Fluid Protocol Procedure

### *1. 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** to the sample mixture and shake vigorously. Incubate at 60°C 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 5 seconds. Incubate at 60°C for at least 20 minutes to ensure the lysate is clear. During incubation, invert the tube every 5 minutes.

**NOTE!** It is essential that the sample and GSB Buffer are mixed thoroughly to yield a homogenous solution. Inverting the sample occasionally during incubation will facilitate Proteinase K digestion and cell lysis. Using an auto shaking system is more convenient when incubating samples. 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).

### *Optional RNA Removal Step*

For RNA-free gDNA, following GSB Buffer addition and 60°C 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 and 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 for 3 minutes at 14-16,000 x g to dry the column matrix.

**NOTE!** Additional centrifugation at 14-16,000 x g for 5 minutes or incubation at 60°C for 5 minutes will completely dry the GS Column to avoid any residual ethanol carryover and ensure the most effective downstream applications.

### *5. Elution*

Standard elution volume is 100 µl. If less sample is to be used, reduce the elution volume (30-50 µl) to increase DNA concentration. If higher DNA yield is required, repeat the DNA Elution step to increase DNA recovery and the total elution volume to approximately 200 µl.

Transfer the dried **GS Column** to a clean 1.5 ml microcentrifuge tube. Add **100 µl of pre-heated Elution Buffer**[1], TE Buffer[2] or water[3] into the **CENTER** of the column matrix. Incubate the **GS Column** at 37°C for 10 minutes. Centrifuge at 14-16,000 x g for 1 minute to elute the purified DNA.

[1]Ensure that Elution Buffer (10 mM Tris-HCl, pH8.5 at 25°C) is added into the center of the GS Column matrix and is completely absorbed.

[2]Using TE (10 mM Tris-HCl, 1 mM EDTA, pH8.0) for elution is beneficial as EDTA preserves DNA for longterm storage. However, EDTA will affect PCR and other sensitive downstream applications. Ensure that TE is added into the center of the GS Column matrix and is completely absorbed.

[3]If using water for elution, ensure the water pH is between 7.0 and 8.5. dd$H_2O$ should be fresh as ambient $CO_2$ can quickly cause acidification. Ensure that water is added into the center of the GS Column matrix and is completely absorbed. DNA Eluted in water should be stored at -20°C to avoid degradation.

# FFPE Protocol Procedure

### *1. 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.

**NOTE!** Inverting the sample occasionally during incubation will facilitate Proteinase K digestion and cell lysis. Using an auto shaking system is more convenient when incubating samples. 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).

### *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** then 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. Wash*

Add **400 µl of W1 Buffer** to the **GS Column**. Centrifuge at 14-16,000 x g for 30 seconds. 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 for 3 minutes at 14-16,000 x g to dry the column matrix.

**NOTE!** Additional centrifugation at 14-16,000 x g for 5 minutes or incubation at 60oC for 5 minutes will completely dry the GS Column to avoid any residual ethanol carryover and ensure the most effective downstream applications.

### *5. Elution*

Standard elution volume is 100 µl. If less sample is to be used, reduce the elution volume (30-50 µl) to increase DNA concentration. If higher DNA yield is required, repeat the DNA Elution step to increase DNA recovery and the total elution volume to approximately 200 µl.

Transfer the dried **GS Column** to a clean 1.5 ml microcentrifuge tube. Add **100 µl of pre-heated Elution Buffer[1]**, TE Buffer[2] or water[3] 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 the purified DNA.

[1]Ensure that Elution Buffer (10 mM Tris-HCl, pH8.5 at 25°C) is added into the center of the GS Column matrix and is completely absorbed.

[2]Using TE (10 mM Tris-HCl, 1 mM EDTA, pH8.0) for elution is beneficial as EDTA preserves DNA for longterm storage. However, EDTA will affect PCR and other sensitive downstream applications. Ensure that TE is added into the center of the GS Column matrix and is completely absorbed.

[3]If using water for elution, ensure the water pH is between 7.0 and 8.5. dd$H_2O$ should be fresh as ambient $CO_2$ can quickly cause acidification. Ensure that water is added into the center of the GS Column matrix and is completely absorbed. DNA Eluted in water should be stored at -20°C to avoid degradation.

# 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 60°C for 30 minutes to lyse the sample. During incubation, invert the tube every 5 minutes.

**NOTE!** IInverting the sample occasionally during incubation will facilitate Proteinase K digestion and cell lysis. Using an auto shaking system is more convenient when incubating samples. 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 4 DNA Elution).

Add **200 µl of GSB Buffer** and mix vigorously. Incubate at 60°C for 20 minutes. During incubation, invert the tube every 5 minutes.

**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 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. During centrifugation, place a **GS Column in a 2 ml Collection Tube**. Following centrifugation, transfer the supernatant to a new 1.5 ml microcentrifuge tube.

### *2. DNA Binding*

Add **200 µl of absolute ethanol** to the sample lysate then mix **IMMEDIATELY** by shaking vigorously for 10 seconds. If precipitate appears, break it up as much as possible with a pipette. Transfer **all of the sample mixture (including any insoluble precipitate) to the GS Column** then 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.

### *3. Wash*

Add **400 µl of W1 Buffer** to the **GS Column**. Centrifuge at 14-16,000 x g for 30 seconds. 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 for 3 minutes at 14-16,000 x g to dry the column matrix.

**NOTE!** Additional centrifugation at 14-16,000 x g for 5 minutes or incubation at 60°C for 5 minutes will completely dry the GS Column to avoid any residual ethanol carryover and ensure the most effective downstream applications.

### *4. Elution*

Standard elution volume is 100 µl. If less sample is to be used, reduce the elution volume (30-50 µl) to increase DNA concentration. If higher DNA yield is required, repeat the DNA Elution step to increase DNA recovery and the total elution volume to approximately 200 µl.

Transfer the dried **GS Column** to a clean 1.5 ml microcentrifuge tube. Add 100 µl of pre-heated Elution Buffer[1], TE Buffer[2] or water[3] 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 the purified DNA.

[1]Ensure that Elution Buffer (10 mM Tris-HCl, pH8.5 at 25°C) is added into the center of the GS Column matrix and is completely absorbed.

[2]Using TE (10 mM Tris-HCl, 1 mM EDTA, pH8.0) for elution is beneficial as EDTA preserves DNA for longterm storage. However, EDTA will affect PCR and other sensitive downstream applications. Ensure that TE is added into the center of the GS Column matrix and is completely absorbed.

[3]If using water for elution, ensure the water pH is between 7.0 and 8.5. dd$H_2O$ should be fresh as ambient $CO_2$ can quickly cause acidification. Ensure that water is added into the center of the GS Column matrix and is completely absorbed. DNA Eluted in water should be stored at -20°C to avoid degradation.

# Insect Protocol Procedure

### *1. 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.

**NOTE!** Inverting the sample occasionally during incubation will facilitate Proteinase K digestion and cell lysis. Using an auto shaking system is more convenient when incubating samples. 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).

### *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** then 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.Wash*

Add **400 µl of W1 Buffer** to the **GS Column**. Centrifuge at 14-16,000 x g for 30 seconds. 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 for 3 minutes at 14-16,000 x g to dry the column matrix.

**NOTE!** Additional centrifugation at 14-16,000 x g for 5 minutes or incubation at 60°C for 5 minutes will completely dry the GS Column to avoid any residual ethanol carryover and ensure the most effective downstream applications.

### *5. Elution*

Standard elution volume is 100 µl. If less sample is to be used, reduce the elution volume (30-50 µl) to increase DNA concentration. If higher DNA yield is required, repeat the DNA Elution step to increase DNA recovery and the total elution volume to approximately 200 µl.

Transfer the dried **GS Column** to a clean 1.5 ml microcentrifuge tube. Add **100 µl of pre-heated Elution Buffer**[1], TE Buffer[2] or water[3] 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 the purified DNA.

[1]Ensure that Elution Buffer (10 mM Tris-HCl, pH8.5 at 25°C) is added into the center of the GS Column matrix and is completely absorbed.

[2]Using TE (10 mM Tris-HCl, 1 mM EDTA, pH8.0) for elution is beneficial as EDTA preserves DNA for longterm storage. However, EDTA will affect PCR and other sensitive downstream applications. Ensure that TE is added into the center of the GS Column matrix and is completely absorbed.

[3]If using water for elution, ensure the water pH is between 7.0 and 8.5. dd$H_2O$ should be fresh as ambient $CO_2$ can quickly cause acidification. Ensure that water is added into the center of the GS Column matrix and is completely absorbed. DNA Eluted in water should be stored at -20°C to avoid degradation.

# Sperm Protocol Procedure

### *1. 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 IBI Scientific.

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.

**NOTE!** Inverting the sample occasionally during incubation will facilitate Proteinase K digestion and cell lysis. Using an auto shaking system is more convenient when incubating samples. 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).

### *2 . Cell Lysis*

Add 200 µl of GSB Buffer then 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.Wash*

Add **400 µl of W1 Buffer** to the **GS Column**. Centrifuge at 14-16,000 x g for 30 seconds. 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 for 3 minutes at 14-16,000 x g to dry the column matrix.

**NOTE!** Additional centrifugation at 14-16,000 x g for 5 minutes or incubation at 60°C for 5 minutes will completely dry the GS Column to avoid any residual ethanol carryover and ensure the most effective downstream applications.

### *5. Elution*

Standard elution volume is 100 µl. If less sample is to be used, reduce the elution volume (30-50 µl) to increase DNA concentration. If higher DNA yield is required, repeat the DNA Elution step to increase DNA recovery and the total elution volume to approximately 200 µl.

Transfer the dried **GS Column** to a clean 1.5 ml microcentrifuge tube. Add **100 µl of pre-heated Elution Buffer**[1], TE Buffer[2] or water[3] 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 the purified DNA.

[1]Ensure that Elution Buffer (10 mM Tris-HCl, pH8.5 at 25°C) is added into the center of the GS Column matrix and is completely absorbed.

[2]Using TE (10 mM Tris-HCl, 1 mM EDTA, pH8.0) for elution is beneficial as EDTA preserves DNA for longterm storage. However, EDTA will affect PCR and other sensitive downstream applications. Ensure that TE is added into the center of the GS Column matrix and is completely absorbed.

[3]If using water for elution, ensure the water pH is between 7.0 and 8.5. $ddH_2O$ should be fresh as ambient $CO_2$ can quickly cause acidification. Ensure that water is added into the center of the GS Column matrix and is completely absorbed. DNA Eluted in water should be stored at -20°C to avoid degradation.

# Troubleshooting

## Low Yield

**Incomplete buffer preparation.**

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. Fresh blood is recommended. However, frozen or blood treated with anticoagulants can also be used. Increased storage length decreases DNA yield. If using nucleated blood (e.g. bird or fish) use up to 10 µl then adjust volume to 200 µl with PBS. Hair samples should completely submerged in GST Buffer and Proteinase K.

**Incomplete buffer preparation.**

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. Add $ddH_2O$ pH7.0-8.5 (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 $ddH_2O$ and Proteinase K mixture should be stored at 4°C. Use only fresh $ddH_2O$ as ambient $CO_2$ can quickly cause acidification.

**Incorrect DNA elution step.**

Ensure that Elution Buffer, TE or water is added into the **CENTER** of the GS Column matrix and is completely absorbed. Use pre-heated Elution Buffer, TE, or water (60~70°C). If using water for elution, ensure the water pH is between 7.0 and 8.5. $ddH_2O$ should be fresh as ambient $CO_2$ can quickly cause acidification. Elute twice to increase the DNA recovery.

**Residual ethanol contamination.**

Following the wash step, dry the GS Column with additional centrifugation at 14-16,000 x g for 5 minutes to ensure the GS Column mebrane is completely dry.

**Clogged column.**

Use the recommended amount of starting material or separate into multiple tubes. Add additional Proteinase K and extend the incubation time in the Lysis Step. Following the Lysis Step, centrifuge for 2 minutes at 14-16,000 x g to remove sample debris. Transfer the supernatant to a new microcentrifuge tube and proceed with the DNA Binding Step. If precipitate formed at the DNA Binding Step reduce the sample material. Following ethanol addition, break up any precipitate as much as possible prior to loading GS Column.

## Eluted DNA Does Not Perform Well In Downstream Applications

**Residual ethanol contamination.**

Following the wash step, dry the GS Column with additional centrifugation at 14-16,000 x g for 5 minutes to ensure the GS Column membrane is completely dry.

**Residual RNA Contamination.**

Perform the optional RNA removal step.

# The gMAX DNA Mini Kit Functional Test Data

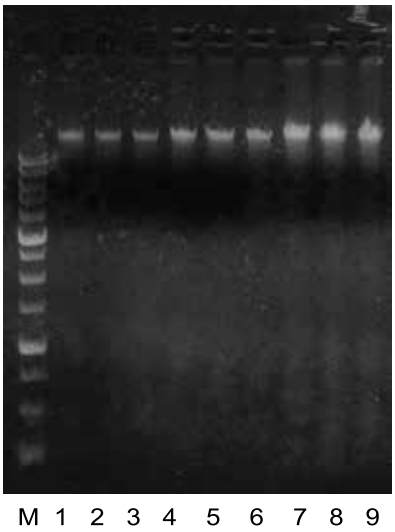



**Figure 1.**

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

1-3 = 50 µl whole blood sample

4-6 = 100 µl whole blood sample

7-9 = 200 µl whole blood sample

M = 1 Kb DNA Ladder

| Volume | Yield | 260/280 |
|---|---|---|
| 50 µl | 1.54 µg | 1.85 |
| 100 µl | 2.70 µg | 1.87 |
| 200 µl | 5.56 µg | 1.90 |

# Related DNA/RNA Extraction Products

| Total RNA Extraction | | |
|---|---|---|
| Product | Package size | Catalogue number |
| Total RNA Mini Kit (Blood/Cell) | 50/100/300 preps | IB47321/322/323 |
| Total RNA Mini Kit (Tissue) | 50/100/300 preps | IB47301/302/303 |
| Total RNA Maxi Kit (Plant) | 50/100/300 preps | IB47341/342/343 |
| rBAC Mini RNA Bacteria Kit | 100/300 preps | IB47421/422 |
| rYeast Total RNA Mini Kit | 100/300 preps | IB47411/412 |
| miRNA Isolation Kit | 100 preps | IB47371 |
| IBI Isolate | 100/200 rxns | IB47601/302 |
| **Plasmid DNA Purification** | | |
| Product | Package size | Catalogue number |
| I-Blue Mini Plasmid Kit | 100/300 preps | IB47171/172 |
| I-Blue Midi Plasmid Endo Free Kit | 100 preps | IB47176 |
| I-Blue Midi Plasmid Kit | 25 preps | IB47181 |
| I-Blue Midi Plasmid Kit (Endotoxin Free) | 25 preps | IB47191 |
| Midi fast Ion Plasmid Midi Kit | 25 preps | IB47111 |
| Midi fast Ion Plasmid Kit (Endotoxin Free) | 25 preps | IB47113 |
| Maxi fast Ion Plasmid Midi Kit | 10/25 preps | IB47121/122 |
| Maxi fast Ion Plasmid Kit (Endotoxin Free) | 10/25 preps | IB47124/125 |
| 96-Well Plasmid Kit | 4/10 x 96 preps | IB47151/152 |
| **Post Reaction DNA Extraction** | | |
| Product | Package size | Catalogue number |
| Gel/PCR DNA Fragments Extraction Kit | 100/300 preps | IB47020/030 |
| Small DNA Fragments Extraction Kit | 100/300 preps | IB47061/062 |
| 96 Well PCR Cleanup Kit | 4/10 x 96 preps | IB47040/050 |
| **Genomic DNA Extraction** | | |
| Product | Package size | Catalogue number |
| Genomic DNA Mini Kit (Blood/Cultured Cell) | 100/300 preps | IB47201/202 |
| Genomic DNA Mini Kit (Tissue) | 50/300 preps | IB47221/222 |
| gMAX Mini Kit (Blood/Tissue) | 100/300 preps | IB47281/282 |
| Genomic DNA Mini Kit (Plant) | 100 preps | IB47230 |
| gSWAB Mini Genomic DNA Kit | 100/300 preps | IB47276/277 |
| gBAC Mini DNA Bacteria Kit | 100/300 preps | IB47291/292 |
| gYEAST Genomic DNA Kit | 100/300 preps | IB47266/267 |
| 96 Well Blood Genomic DNA Extraction Kit | 4/10 x 96 preps | IB47251/252 |
| gPURE Cell DNA Isolation Kit | 100/1000 rxns | IB47431/432 |
| **DNA/RNA/Protein Extraction** | | |
| Product | Package size | Catalogue number |
| DNA/RNA/Protein Extraction Kit | 50/100 preps | IB47701/702 |

For additional product information please visit **www.ibisci.com** Thank you!

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