IBI SCIENTIFIC

Instruction Manual
Ver. 02.10.17
For Research Use Only

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, buffy coat, body fluids, cultured cells, amniotic fluid, 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)

Table of Contents

Introduction.......... 2
Quality Control.......... 2
Kit Components.......... 2
Safety Measures.......... 3
Quick Protocol Diagram.......... 3
Solid Tissue Protocol.......... 4
Blood, Plasma, Serum, Buffy Coat and Body Fluids Protocol.......... 6
Cultured Cell Protocol.......... 7
Amniotic Fluid Protocol.......... 9
Formalin Fixed Paraffin Embedded Tissue Protocol.......... 10
Hair Protocol.......... 12
Insect Protocol.......... 14
Sperm Protocol.......... 15
Troubleshooting.......... 17
Test Data.......... 18
Related Products.......... 18

IBI Scientific
Tel: 1 800 253 4942 · Fax: 563 690 0490 · www.ibisci.com · info@ibisci.com

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 μ l 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

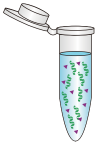
Component	IB47280	IB47281	IB47282
GST Buffer	3 ml	30 ml	75 ml
GSB Buffer	4 ml	40 ml	75 ml
W1 Buffer	2 ml	45 ml	130 ml
Wash Buffer[1] (Add Ethanol)	1 ml (4 ml)	25 ml (100 ml)	50 ml (200 ml)
Proteinase K[2] (Add ddH_2O)	1 mg (0.10 ml)	11 mg (1.10 ml)	65 mg (6.50 ml)
Elution Buffer	1 ml	30 ml	75 ml
GS Columns	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.

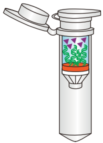
[2]Add ddH_2O to Proteinase K (see the bottle label for volume) then vortex to ensure it is completely dissolved. Check the box on the bottle. Once it is dissolved completely, centrifuge for a few seconds to spin the mixture down. For extended periods, the ddH_2O and Proteinase K mixture should be stored at 4ºC. 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.

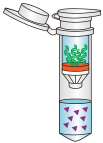
Quick Protocol Diagram



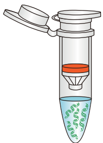
Sample preparation and cell lysis



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. 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 be stored at 4ºC. Use only fresh ddH_2O as ambient CO_2 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 -20ºC or -70ºC 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 60ºC (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 25ºC) 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. 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 -20ºC 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 60ºC 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 60ºC 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 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 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 25ºC) 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. 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 -20ºC 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 60ºC for 5 minutes.

2. Cell Lysis

Add **200 µl of GSB Buffer** then mix by shaking vigorously. Incubate at 60ºC 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 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.

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 25ºC) 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. 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 -20ºC 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 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 5 seconds. Incubate at 60ºC 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 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 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. 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. Incubate the **GS Column** at 37ºC 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 25ºC) 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. 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 -20ºC 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 37ºC for 15 minutes to evaporate ethanol residue.

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: 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 60ºC (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 25ºC) 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. 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 -20ºC 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 60ºC for 30 minutes to lyse the sample. During incubation, invert the tube every 5 minutes.

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 60ºC (for Step 4 DNA Elution).

Add **200 µl of GSB Buffer** and mix vigorously. Incubate at 60ºC 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 60ºC 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 25ºC) 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. 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 -20ºC 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 60ºC 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 60ºC (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 25ºC) 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. 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 -20ºC 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 -20ºC. 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 60ºC 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 60ºC (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 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 25ºC) 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. 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 -20ºC to avoid degradation.

Troubleshooting

Low Yield

Improper sample homogenization.

Yield and quality of DNA will be higher when fresh samples or samples which have been flash frozen and stored at -20ºC or -70ºC 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 4ºC. 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 (60~70ºC). 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

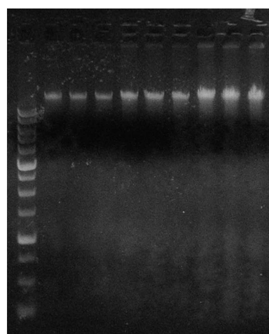


Figure 1. Genomic DNA from 50, 100 and 200 µl whole blood samples was extracted using the gMAX DNA Mini Kit. 10 µl from 100 µl eluates of purified genomic DNA was analyzed by electrophoresis on a 0.8% agarose gel.
1-3 = 50 µl whole blood sample
4-6 = 100 µl whole blood sample
7-9 = 200 µl whole blood 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 Mini 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 Extraction		
Product	Package Size	Catalogue Number
I-Blue Mini Plasmid Kit	100/300 preps	IB47171/172
I-Blue Mini 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 Kit	25 preps	IB47111
Midi Fast Ion Plasmid Kit (Endotoxin Free)	25 preps	IB47113
Maxi Fast Ion Plasmid 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!

IBI SCIENTIFIC

www.ibisci.com