Geneaid

Instruction Manual
Ver. 09.14.23
For Research Use Only

gSYNC™ DNA Extraction Kit

GS004 (4 Preparation Sample Kit)
GS100 (100 Preparation Kit)
GS300 (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)

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Introduction

The gSYNC™ DNA Extraction 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 gSYNC™ DNA Extraction 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

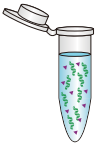
Component	GS004	GS100	GS300
GST Buffer	3 ml	30 ml	75 ml
GSB Buffer	4 ml	40 ml	75 ml
W1 Buffer	2 ml	45 ml	130 ml
Wash Buffer[1] (Add Ethanol)	1 ml (4 ml)	25 ml (100 ml)	50 ml (200 ml)
Proteinase K[2] (Add ddH_2O)	1 mg (0.10 ml)	11 mg x 2 (1.10 ml)	65 mg (6.50 ml)
Elution Buffer	1 ml	30 ml	75 ml
GS Columns	4	100	300
2 ml Collection Tubes	8	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.

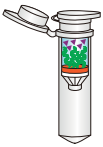
[2]Add ddH_2O to Proteinase K (see the bottle label for volume) then vortex to ensure it is completely dissolved. Check the box on the bottle. Once it is dissolved completely, centrifuge for a few seconds to spin the mixture down. For extended periods, the ddH_2O and Proteinase K mixture should be stored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

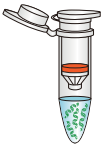


Sample preparation and cell lysis



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

gSYNC™ 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 4-10 FFPE sections of 5-10 µm thick and transfer them to a 1.5 ml microcentrifuge tube. Using a sterile blade is recommended. Add **1 ml of xylene** then vortex vigorously for 10 seconds. 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 vortexing. Centrifuge at 14-16,000 x g for 3 minutes then remove the supernatant. Carefully remove any residual ethanol using a fine pipet tip. Open the tube and incubate at 60ºC for 3-10 minutes to evaporate ethanol residue.

Add **200 µl of GST Buffer and 20 µl of Proteinase K** then vortex thoroughly. Incubate at 60ºC for 1 hour 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.

Set heating block to 90ºC and incubate the sample lysate at 90°C for 1 hour.

NOTE: If using only one heating block, leave the sample at room temperature after the 60°C incubation until the heating block has reached 90°C. The incubation at 90°C partially reverses formaldehyde modification of nucleic acids. Longer incubation times or higher incubation temperatures may result in more fragmented DNA.

NOTE: 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 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 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

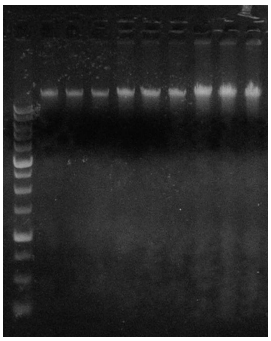
Residual ethanol contamination.

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 gSYNC™ DNA Extraction Kit Functional Test Data



M 1 2 3 4 5 6 7 8 9

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.
1-3 = 50 µl whole blood sample
4-6 = 100 µl whole blood sample
7-9 = 200 µl whole blood sample
M = Geneaid 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

Genomic DNA Extraction		
Product	Package Size	Catalogue Number
Genomic DNA Mini Kit (Blood/Cultured Cell)	100/300 preps	GB100/300
Genomic DNA Maxi Kit (Blood/Cultured Cell)	10/25 preps	GDM10/25
Genomic DNA Mini Kit (Tissue)	50/100/300 preps	GT050/100/300
gSYNC™ DNA Extraction Kit	50/100/300 preps	GS050/100/300
Genomic DNA Mini Kit (Plant)	100 preps	GP100
Genomic DNA Maxi Kit (Plant)	10/25 preps	GPM010/25
GENEzol™ DNA Reagent Plant	100/200 rxns	GR100/200
Presto™ Mini gDNA Yeast Kit	100/300 preps	GBY100/300
Presto™ Mini gDNA Bacteria Kit	100/300 preps	GBB100/101/300/301
Geneius™ Micro DNA Extraction Kit	100/300 preps	GMB100/300
Presto™ Buccal Swab gDNA Extraction Kit	100/300 preps	GSK100/300
Presto™ 96 Well Blood Genomic DNA Extraction Kit	4/10 x 96 preps	96GBP04/10
Presto™ 96 Well Plant Genomic DNA Extraction Kit	4/10 x 96 preps	96GPP04/10
DNA RNA Purification		
Product	Package Size	Catalogue Number
Presto™ DNA/RNA Extraction Kit	50/100 preps	DR050/100
Presto™ DNA/RNA/Protein Extraction Kit	50/100 preps	DRP050/100

Related DNA/RNA Extraction Products

RNA Extraction and Purification		
Product	Package Size	Catalogue Number
Total RNA Mini Kit (Blood/Cultured Cell)	50/100/300 preps	RB050/100/300
Total RNA Mini Kit (Tissue)	50/100/300 preps	RT050/100/300
Total RNA Mini Kit (Plant)	50/100/300 preps	RP050/100/300
Presto™ Mini RNA Bacteria Kit	50/100/300 preps	RBB050/100/300
Presto™ Mini RNA Yeast Kit	50/100/300 preps	RBY050/100/300
miRNA Isolation Kit	50/100 preps	RMI050/100
GENEzol™ Reagent	50/100/200 rxns	GZR050/100/200
GENEzol™ TriRNA Bacteria Kit	50/100 rxns	GZB050/100
GENEzol™ TriRNA Pure Kit	50/100/200 preps	GZX050/100/200
TriRNA Pure Kit	50/100/200 preps	TRP050/100/200
RNA Pure Kit	50/100 preps	PR050/100
Virus DNA/RNA Purification		
Product	Package Size	Catalogue Number
Plant Virus RNA Kit	50/100 preps	PVR050/100
Viral Nucleic Acid Extraction Kit II	50/100/300 preps	VR050/100/300
Viral Nucleic Acid Extraction Kit III	50/100/300 preps	VI050/100/300
Plasmid DNA Purification		
Product	Package Size	Catalogue Number
Presto™ Mini Plasmid Kit	100/300 preps	PDH100/300
Presto™ Midi Plasmid Kit	25 preps	PIF025
Presto™ Midi Plasmid Kit (Endotoxin Free)	25 preps	PIFE25
High-Speed Plasmid Mini Kit (10-50 Kb)	100/300 preps	PDL100/300
High-Speed Plasmid Advance Kit (50-100 ml)	25 preps	PA025
Geneaid™ Midi Plasmid Kit	25 preps	PI025
Geneaid™ Midi Plasmid Kit (Endotoxin Free)	25 preps	PIE25
Presto™ Plasmid DNA Concentration Kit	250/500/1000 preps	PC0250/500/1000
Geneaid™ Maxi Plasmid Kit	10/25 preps	PM010/25
Geneaid™ Maxi Plasmid Kit (Endotoxin Free)	10/25 preps	PME10/25
Presto™ 96 Well Plasmid Kit	4/10 x 96 preps	96PDV04/10, 96PDC04/10
Post Reaction DNA Purification		
Product	Package Size	Catalogue Number
GenepHlow™ Gel Extraction Kit	100/300 preps	DFG100/300
GenepHlow™ PCR Cleanup Kit	100/300 preps	DFC100/300
GenepHlow™ Gel/PCR Kit	100/300 preps	DFH100/300
GenepHlow™ DNA Cleanup Midi Kit	100/300 preps	DFI100/300
GenepHlow™ DNA Cleanup Maxi Kit	10/25 preps	DFM010/025
Small DNA Fragments Extraction Kit	100/300 preps	DF101/301
Presto™ Max Gel/PCR Kit (Large DNA Fragments)	100/300 preps	DFL100/300
Presto™ 96 Well PCR Cleanup Kit	4/10 x 96 preps	96DFH04/10
Presto™ 96 Well Gel Extraction Kit	4/10 x 96 preps	96DFG04/10
G-25 Gel Filtration Desalting Column	50 rxns	CG025
G-50 Gel Filtration Dye Terminator Removal Column	50 rxns	CG050
96-Well G-50 Gel Filtration Plate	4/10 x 96 rxns	CGP04/10

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

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