gSYNC™ DNA Extraction Kit Quick Protocol

For research use only

Catalogue Number

GS004, GS100, GS300

Instruction Manual Download

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

IMPORTANT BEFORE USE!

1. Add absolute ethanol (see the bottle label for volume) to Wash Buffer then mix by shaking for a few seconds. Check the box on the bottle. Be sure and close the bottle tightly after each use to avoid ethanol evaporation.
2. Add 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. 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.
4. Optionally prepare RNase A (50 mg/ml) for RNA-free DNA when performing sensitive downstream reactions. However, residual RNA will not affect PCR.

Tissue, FFPE, Insect and Sperm Protocol Procedure

1A. Tissue Sample Dissociation

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

1B. FFPE Sample Preparation

Cut **4-10 FFPE sections of 5-10 µm thick** and transfer the sections to a 1.5 ml microcentrifuge tube. 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. 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. Set heating block to 90 ºC and incubate the sample lysate at 90°C for 1 hour. (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). Heat Elution Buffer (200 µl/sample) to 60ºC for Step 5 DNA Elution.

1C. Insect Sample Preparation

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

1D. Sperm Sample Preparation

Add ddH_2O to DTT powder (see the bottle label for volume) then vortex to dissolve. (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. During incubation, invert the tube occasionally and transfer the required volume of Elution Buffer (200 µl/sample) to a 1.5 ml microcentrifuge tube and heat to 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** and shake vigorously for 10 seconds.

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

Optional RNA Removal Step

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

3. DNA Binding

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

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

4. Wash

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

5. DNA Elution

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

Hair Protocol Procedure

1. Cell Lysis

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

Optional RNA Removal Step

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

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

2. DNA Binding

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

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

3. Wash

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

4. Elution

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

Blood, Cultured Cell, Amniotic Fluid Protocol Procedure

1A. Blood Sample Preparation

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

1B. Cultured Cell Sample Preparation

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

1C. Amniotic Fluid Sample Preparation

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

2. Cell Lysis

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

Optional RNA Removal Step

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

3. DNA Binding

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

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

4. Wash

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

5. Elution

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

Kit Components

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 pH7.0-8.5 (see the bottle label for volume) to Proteinase K then vortex to ensure Proteinase K is completely dissolved. Check the box on the bottle. Once it is dissolved completely, centrifuge for a few seconds to spin down the mixture. For extended periods, the ddH_2O and Proteinase K mixture should be stored at 4ºC. Use only fresh ddH_2O as ambient CO_2 can quickly cause acidification.

The gSYNC™ DNA Extraction Kit Functional Test Data

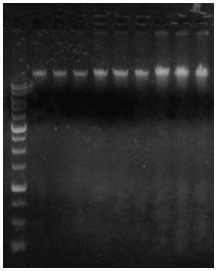

Figure 1. Genomic DNA from 50, 100 and 200 µl whole blood samples was extracted using the gSYNC™ DNA Extraction Kit. 10 µl from 100 µl eluates of purified genomic DNA was analyzed by electrophoresis on a 0.8% agarose gel.
1-3 = 50 µl whole blood sample
4-6 = 100 µl whole blood sample
7-9 = 200 µl whole 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

Storage: dry at room temperature (15-25ºC)