gSYNC™ 96 Well DNA Extraction Kit Quick Protocol

For research use only

Catalogue Number

96GS002, 96GS004, 96GS010,

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.

Instruction Manual Download

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. 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. 96 Deep Well Plates are reusable. After use, rinse plates with water then incubate in 0.4M HCl for 1 minute at room temperature. Wash plates thoroughly with ddH_2O. Autoclave plates after being washed.

4. Yield and quality of DNA will be higher when fresh samples or flash frozen (-20ºC or -70ºC) samples are used. DNA in tissue which has been repeatedly frozen and thawed may be degraded.

Proteinase K Working Solution Preparation

A. Mix **20 µl of Proteinase K and 180 µl of GST Buffer** per sample by vortex.

B. For 96 samples, mix **2 ml of Proteinase K and 18 ml of GST Buffer** by vortex.

Binding Solution Preparation

A. Mix **200 µl of GSB Buffer and 200 µl of absolute ethanol** per sample by shaking.

B. For 96 samples, mix **20 ml of GSB Buffer and 20 ml of absolute ethanol** by shaking.

Centrifuge Protocol

1. Tissue Dissociation and Lysis (Centrifuge and Vacuum protocol)

Transfer up to **20 mg of animal tissue (0.5 cm mouse tail) or 1 x 10^6 cultured cells** into each tube of **Microtubes (Racked)**. Add **200 µl of Proteinase K working solution** to each microtube. Seal the tubes with the **Caps for Microtubes (8-strip)** then cover the rack with the plastic cover and mix by inverting. Briefly centrifuge at 3,000 x g to collect any solution from the caps. Allow the centrifuge to reach 3,000 x g prior to stopping. It's important that the tissue samples are completely immersed in Proteinase K working solution. Incubate at 60ºC until the sample lysate becomes clear (at least 6 hours or 10 minutes for cultured cells). Place a weight on top of the plastic cover during incubation. For optimal lysis, mix occasionally or place the rack on a rocking platform during incubation. After incubation, make sure the microtubes are sealed properly. Cover the rack with the plastic cover then mix the sample lysate by shaking vigorously for 10 seconds. Briefly centrifuge at 3,000 x g to collect any solution from the caps. Allow the centrifuge to reach 3,000 x g prior to stopping.

Optional RNA removal step: For RNA-free gDNA, add 5 µl of RNase A (10 mg/ml) into each microtube. Seal the tubes with new caps (not provided). Cover the rack with the plastic cover then mix by shaking vigorously. Briefly centrifuge at 3,000 x g to collect any solution from the caps. Allow the centrifuge to reach 3,000 x g prior to stopping. Incubate for 5 minutes at room temperature.

NOTE: During incubation, pre-heat the required volume of Elution Buffer (400 µl/sample) to 60ºC for step 4 DNA Elution.

2. DNA Binding

Carefully open **Microtubes (Racked)** and add **400 µl of Binding Solution** to each microtube. Seal the microtubes with new caps. Cover the rack with the plastic cover then mix by shaking vigorously for 15-30 seconds. Briefly centrifuge at 3,000 x g to collect any lysate from the caps. Allow the centrifuge to reach 3,000 x g prior to stopping. Place a **Presto™ gDNA 96 Well Binding Plate** on a new **96 Deep Well Plate**. Carefully open microtubes and transfer all of the sample lysate into each well being careful not to get any lysate on the rims of the wells. DO NOT transfer insoluble particles such as hair or bones to the **96 Well Binding Plate** to prevent membrane clogging. Centrifuge the **96 Well Binding Plate** and **96 Deep Well Plate** together at 3,000 x g for 5 minutes. Discard the flow-through then place the **96 Well Binding Plate** back on the **96 Deep Well Plate**.

3. Wash

Add **400 µl of W1 Buffer** to each well of the **Presto™ gDNA 96 Well Binding Plate** then centrifuge together with the **96 Deep Well Plate** at 3,000 x g for 5 minutes. Discard the flow-through then place the **96 Well Binding Plate** back on the **96 Deep Well Plate**. Add **600 µl of Wash Buffer (make sure ethanol was added)** to each well then centrifuge together with the **96 Deep Well Plate** at 3,000 x g for 5 minutes. Discard the flow-through. Place the **96 Well Binding Plate** back on the **96 Deep Well Plate** and centrifuge at 3,000 x g for 10 minutes to dry the membrane.

4. Elution

Remove the **Presto™ gDNA 96 Well Binding Plate** from the **96 Deep Well Plate** then blot the nozzles on a clean absorbent paper towel to remove residual ethanol. Place the **96 Well Binding Plate** on top of **Microtubes (Racked)**. Add **200 µl of pre-heated Elution Buffer**, TE or water into the CENTER of each well. Let stand for at least 2 minutes to ensure the Elution Buffer, TE or water is absorbed by the membrane. Centrifuge the **96 Well Binding Plate** and the **Microtubes (Racked)** together at 3,000 x g for 5 minutes. Seal the microtubes with new caps and store the DNA at -20ºC.

Vacuum Protocol

1. Vacuum Manifold Preparation

Set the waste tray in the manifold base. Place the binding top plate on the manifold base. Place a **Presto™ gDNA 96 Well Binding Plate** in the binding top plate aperture. Attach the manifold to a vacuum source.

2. Tissue Dissociation and Lysis

See Page 1 of 4.

3. DNA Binding

Carefully open **Microtubes (Racked)** and add **400 µl of Binding Solution** to each microtube. Seal the microtubes with new caps. Cover the rack with the plastic cover then mix by shaking vigorously for 15-30 seconds. Briefly centrifuge at 3,000 x g to collect any lysate from the caps. Allow the centrifuge to reach 3,000 x g prior to stopping. Carefully open microtubes and transfer all of the sample lysate into each well of the **Presto™ gDNA 96 Well Binding Plate** being careful not to get any lysate on the rims of the wells. DO NOT transfer insoluble particles such as hair or bones to prevent membrane clogging. Seal unused wells with adhesive film. Apply vacuum at **15 inches Hg** until samples pass through completely then switch off the vacuum.

4. Wash

Add **400 µl of W1 Buffer** to each well of the **Presto™ gDNA 96 Well Binding Plate**. Apply vacuum at **15 inches Hg** until **W1 Buffer** passes through completely (approx. 10 seconds). Switch off the vacuum. Add **600 µl of Wash Buffer (make sure ethanol was added)** to each well. Apply vacuum at **15 inches Hg** until **Wash Buffer** passes through completely. Apply vacuum for an additional 10 minutes to dry the membrane then switch off the vacuum.

5. Elution

Remove the **Presto™ gDNA 96 Well Binding Plate** from the **96 Deep Well Plate** then blot the nozzles on a clean absorbent paper towel to remove residual ethanol. Replace the waste tray with Microtubes (Racked). Place the binding top plate on the manifold base then place the **96 Well Binding Plate** in the binding top plate aperture. Add **200 µl of pre-heated Elution Buffer**, TE or water into the CENTER of each well. Let stand for at least 2 minutes to ensure the Elution Buffer, TE or water is absorbed by the membrane. Apply vacuum at **15 inches Hg for 5 minutes** to elute the purified DNA. Seal the microtubes with new caps and store the purified DNA at -20ºC.

Components

Component	96GS002	96GS004	96GS010
GST Buffer	60 ml	100 ml	200 ml
GSB Buffer	60 ml	100 ml	155 ml x 1 60 ml x 1
W1 Buffer	80 ml	200 ml	200 ml x 2
Wash Buffer[1] (Add Ethanol)	25 ml (100 ml)	50 ml (200 ml)	25 ml x 1 (100 ml) 50 ml x 2 (200 ml x 2)
Proteinase K[2] (Add ddH_2O)	11 mg x 4 (1.1 ml x 4)	65 mg x 1 (6.5 ml) 11 mg x 2 (1.1 ml x 2)	55 mg x 4 (5.5 ml x 4)
Elution Buffer	100 ml	100 ml x 2	100 ml x 4
RNase A (10 mg/ml)	1 ml	2 ml	5 ml
Presto™ gDNA 96 Well Binding Plates	2	4	10
Microtubes (Racked)	2	2	2
Microtubes (8-strip)	12 x 2	12 x 6	12 x 18
Caps for Microtubes (8-strip)	72	72 x 2	72 x 5
96 Deep Well Plates[3]	2	2	2

[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.

[3]96 Deep Well Plates are reusable. After use, rinse the plate with water then incubate in 0.4M HCl for 1 minute at room temperature. Wash the plate thoroughly with ddH_2O. The plate can be autoclaved after being washed.

Storage

Dry at room temperature (15-25ºC)

Troubleshooting

Low Yield

Improper sample homogenization and lysis

Add 20 µl of Proteinase K to 180 µl of GST Buffer to prepare Proteinase K working solution per sample. Add 200 µl of Proteinase K working solution to each tissue sample, mix well by inverting. Make sure the tissue is completely immerged in the solution. Incubate the sample at 60ºC overnight.

Incomplete buffer preparation.

Add ddH_2O (see bottle label for volume) to dissolve Proteinase K powder and store at 4ºC. Add absolute ethanol (see bottle label for volume) to Wash Buffer prior to use. Be sure and close the bottle tightly after each use to avoid evaporation.

Incorrect DNA elution step.

Use pre-heated Elution Buffer, TE or water (60 ºC) to elute DNA. Ensure Elution Buffer, TE or water is added into the center of the matrix and is completely absorbed. If using water for elution, ensure the water pH is between 7.5 and 8.5. Elute twice to increase the DNA recovery.

Residual RNA Contamination.

Perform the optional RNA removal step.

Clogged column.

Use the recommended amount of starting material. Large amounts of tissue cannot be completely lysed which can clog the membrane and decrease DNA yield and quality.

Undigested tissue has been transferred into wells of binding plate.

DO NOT transfer bones or hairs to binding plates.