Geneius™ Micro gDNA Kit Quick Protocol

For research use only

Catalogue Number

GMB004, GMB100, GMB300

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 1 ml of Elution Buffer to Carrier RNA then vortex to ensure it is completely dissolved to obtain a working solution of 1 µg/µl. Check the box on the bottle. Once it is dissolved completely, centrifuge for a few seconds to spin the mixture down. Divide the Carrier RNA solution into convenient volumes in several RNase-free 1.5 ml microcentrifuge tubes. The Carrier RNA solution should be stored at -20ºC. Do not freeze and thaw the Carrier RNA solution more than 3 times.

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. 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.

Protocol Procedure

1. S2 Buffer Preparation

Transfer **1 µl of Carrier RNA solution and 200 µl of S2 Buffer** per sample to a RNase-free 1.5 ml microcentrifuge tube and vortex shortly to mix. The mixture is for use in the Lysis step.

2. Cell Lysis

A. Whole Blood

Transfer **1-100 µl of whole blood** to a 1.5 ml microcentrifuge tube. Add **S1 Buffer to a final volume of 200 µl and 20 µl of Proteinase K** (make sure ddH_2O was added) then mix by vortex. Incubate at 60ºC for 5 minutes to lyse the sample. Add **200 µl of S2 Buffer** (make sure Carrier RNA solution was added) and mix by vortex. Incubate at 60ºC for 5 minutes (invert the tube every 2 minutes). 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).

B. Dried Blood Spot

Cut out a 6 mm (1/4 inch) diameter circle from a dried blood spot (Whatman® FTA® Card) with a single-hole paper punch then transfer to a 1.5 ml microcentrifuge tube. Add **200 µl of S1 Buffer and 20 µl of Proteinase K** (make sure ddH_2O was added) then mix by vortex (make sure the FTA® Card is completely immersed in the buffer). Incubate at 60ºC for 30 minutes to lyse the sample. During incubation, vortex the tube every 10 minutes. Add **200 µl of S2 Buffer** (make sure Carrier RNA solution was added) then mix by vortex. Incubate at 60ºC for 20 minutes. During incubation, vortex the tube every 10 minutes and transfer the required volume of Elution Buffer (200 µl/sample) to a 1.5 ml microcentrifuge tube. Heat to 60ºC (for Step 5 DNA Elution). After incubation, briefly centrifuge the tube and transfer the supernatant to a new 1.5 ml microcentrifuge tube.

C. Urine

Transfer **1 ml of urine** to a 1.5 ml microcentrifuge tube then centrifuge at 6,000 x g for 2 minutes. Discard the supernatant then add **500 µl of Elution Buffer** to the pellet and vortex for 5 seconds.

NOTE: If using 2-10 ml of urine samples, transfer to a 15 ml centrifuge tube and centrifuge at 6,000 x g for 2 minutes. Discard the supernatant; add 500 µl of Elution Buffer then vortex for 5 seconds. Transfer the sample to a 1.5 ml microcentrifuge tube.

Centrifuge at 6,000 x g for 2 minutes then discard the supernatant. Add **200 µl of S1 Buffer and 20 µl of Proteinase K** (make sure ddH_2O was added) then mix by vortex for 10 seconds. Incubate at 60ºC for 30 minutes to lyse the sample. During incubation, vortex the tube every 10 minutes. Add **200 µl of S2 Buffer** (make sure Carrier RNA solution was added) then mix by vortex. Incubate at 60ºC for 20 minutes. During incubation, vortex the tube every 10 minutes and transfer the required volume of Elution Buffer (200 µl/sample) to a 1.5 ml microcentrifuge tube then heat to 60ºC (for Step 5 DNA Elution).

3. DNA Binding

Add **200 µl of absolute ethanol** to the sample lysate, mix thoroughly by vortex for 10 seconds. If precipitate appears, break it up as much as possible with a pipette. Place a **GD Column** in a 2 ml Collection Tube. Transfer all of the mixture (including any insoluble precipitate) to the **GD Column**. Centrifuge at 14-16,000 x g for 1 minute. Discard the 2 ml Collection Tube containing the flow-through and place the **GD Column** in a new 2 ml collection tube.

4. Wash

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

Standard elution volume is 100 µl. If less sample is to be used, reduce the elution volume (30-100 µl) to increase DNA concentration.

Transfer the dried **GD 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 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 GD 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 GD 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 GD Column matrix and is completely absorbed. DNA eluted in water should be stored at -20ºC to avoid degradation.

Components

Component	GMB004	GMB100	GMB300
S1 Buffer	3 ml	30 ml	75 ml
S2 Buffer	4 ml	30 ml	75 ml
Carrier RNA[1] (Add Elution Buffer)	1 mg (1 ml)	1 mg (1 ml)	1 mg (1 ml)
Proteinase K[2] (Add ddH_2O)	1 mg (0.10 ml)	11 mg x 2 (1.10 ml)	65 mg (6.50 ml)
W1 Buffer	2 ml	45 ml	130 ml
Wash Buffer[3] (Add Ethanol)	1 ml (4 ml)	25 ml (100 ml)	50 ml (200 ml)
Elution Buffer	6 ml	75 ml	75 ml x 2
GD Columns	4	100	300
2 ml Collection Tubes	8	200	600

[1]Add 1 ml of Elution Buffer to Carrier RNA then vortex to ensure it is completely dissolved to obtain a working solution of 1 µg/µl. Check the box on the bottle. Once it is dissolved completely, centrifuge for a few seconds to spin the mixture down. Divide the Carrier RNA solution into convenient volumes in several RNase-free 1.5 ml microcentrifuge tubes. The Carrier RNA solution should be stored at -20ºC. Do not freeze and thaw the Carrier RNA solution more than 3 times.

[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]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.

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