Presto™ Buccal Swab gDNA Kit Quick Protocol

For research use only

Catalogue Number

GSK004, GSK100, GSK300

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. Store the solution at -20ºC. Do not freeze/thaw the 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 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.

Buccal Swab Protocol Procedure

1. S2 Buffer Preparation

Transfer **1 µl of Carrier RNA solution and 500 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. Sample Collection

Firmly scrape the swab against the inside of each cheek 15-20 times, being sure to cover each cheek entirely. Repeat with multiple swabs based on DNA yield requirement. DNA can be extracted immediately or the swab can be air dried and stored at room temperature for approximately 1 month. For extended periods, store the dried swab at -20ºC.

NOTE: Person(s) providing the buccal cell sample should not eat or drink for at least 30 minutes prior to sample collection and the mouth should be rinsed thoroughly with water to reduce the possibility of contamination. The person collecting the sample should wear protective gloves, being careful not to contact the tip of the swab.

3. Sample Preparation

Place the swab tip in a 1.5 ml microcentrifuge tube and remove it by either cutting or ejecting. Add **500 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 10 minutes. Place a **Filter Column** in a 2 ml Collection Tube. Using tweezers, transfer the swab to the **Filter Column** and set the 1.5 ml microcentrifuge tube aside. Centrifuge at 14-16,000 x g for 2 minutes to collect the remaining sample from the swab. Discard the **Filter Column** and swab then transfer the flow-through (up to 200 µl) in the 2 ml Collection Tube to the 1.5 ml microcentrifuge tube containing the sample mixture.

4. Lysis

Add **500 µl of S2 Buffer** (make sure Carrier RNA solution was added) then vortex IMMEDIATELY. Incubate at 60ºC for 10 minutes. Vortex briefly every 5 minutes.

NOTE: It is essential that the sample and S2 Buffer are mixed thoroughly to yield a homogeneous solution. 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 7 Elution).

5. DNA Binding

Add **500 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. Place a **GD Column** in a 2 ml Collection Tube. Transfer **750 µl of the mixture** (including any insoluble precipitate) to the **GD Column**. Centrifuge at 14-16,000 x g for 1 minute then discard the flow-through. Transfer the remaining lysate mixture to the **GD Column**. Centrifuge at 14-16,000 x g for 1 minute. Following centrifugation, if the mixture did not flow through the **GD Column** membrane, increase the centrifuge time until it passes completely. Discard the 2 ml Collection Tube containing the flow-through then transfer the **GD 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.

6. 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) 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. Centrifuge again for 3 minutes at 14-16,000 x g to dry the column matrix.

7. Elution

Standard elution volume is 100 µl. If less sample is to be used, reduce the elution volume (50-100 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.

Kit Components

Component	GSK004	GSK100	GSK300
S1 Buffer	3 ml	60 ml	165 ml
S2 Buffer	4 ml	60 ml	165 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	30 ml	75 ml
Filter Columns	4	100	300
GD Columns	4	100	300
2 ml Collection Tubes	12	300	900

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[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 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)