Geneaid

Instruction Manual
Ver. 02.10.17
For Research Use Only

Presto™ Buccal Swab gDNA Extraction Kit

GSK004 (4 Preparation Sample Kit)
GSK100 (100 Preparation Kit)
GSK300 (300 Preparation Kit)

Advantages

Sample: buccal cell swabs
Yield: up to 2 µg of pure genomic DNA per swab
Format: genomic DNA spin columns
Time: 20 minutes
Elution Volume: 50-100 µl
Kit Storage: dry at room temperature (15-25°C)

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Geneaid Biotech Ltd.
Tel: 886 2 26960999 · Fax: 886 2 26960599 · www.geneaid.com · info@geneaid.com

Introduction

The Presto™ Buccal Swab gDNA Extraction Kit provides an efficient method for purifying DNA (including genomic, mitochondrial and viral DNA) from buccal cells. Proteinase K and chaotropic salt are used to lyse cells and degrade protein, allowing DNA to bind to the glass fiber matrix of the spin column. Carrier RNA is included with the kit to improve the efficiency of DNA binding to the spin column membrane. Contaminants are removed using W1 Buffer and Wash Buffer (containing ethanol). The purified genomic DNA is eluted by a low salt Elution Buffer, TE or water. High quality genomic DNA can be purified in 20 minutes without phenol/chloroform extraction or alcohol precipitation, with an average DNA yield of 2 µg per buccal swab. Purified DNA, with approximately 20-30 kb, is suitable for use in PCR or other enzymatic reactions.

Quality Control

The quality of the Presto™ Buccal Swab gDNA Extraction Kit is tested on a lot-to-lot basis by isolating genomic DNA from buccal cells. The purified DNA (2 µg with an A260/A280 ratio of 1.8-2.0) is quantified with a spectrophotometer and analyzed by electrophoresis on a 0.8% agarose gel.

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

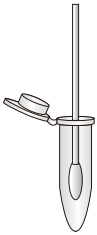
[1]Add 1 ml of Elution Buffer to Carrier RNA then vortex to ensure it is completely dissolved to obtain a working solution of 1 µg/µl. Check the box on the bottle. Once it is dissolved completely, centrifuge for a few seconds to spin the mixture down. Divide the Carrier RNA solution into convenient volumes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

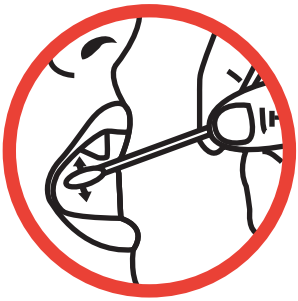
[3]Add absolute ethanol (see the bottle label for volume) to Wash Buffer then mix by shaking for a few seconds. Check the box on the bottle. Be sure and close the bottle tightly after each use to avoid ethanol evaporation.

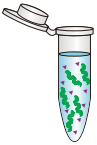
During the procedure, always wear a lab coat, disposable gloves, and protective goggles.

Quick Protocol Diagram

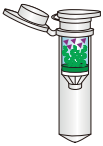


Buccal Cell sample collection



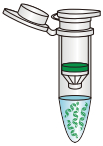


Sample preparation and cell lysis



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

Presto™ Buccal Swab gDNA Extraction Kit Protocol

Please read the entire instruction manual prior to starting the Protocol Procedure.

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 solution should be stored at -20ºC. Do not freeze and 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 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. Close the bottle tightly after each use to avoid ethanol evaporation.

Additional Requirements
Buccal Swabs, RNase-free 1.5 ml microcentrifuge tubes

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.

Troubleshooting

Low Yield

Improper sample collection.

The person providing the buccal cell sample should avoid eating or drinking at least 30 minutes prior to sample collection to avoid contamination. Buccal swabs must be handled with disposable gloves and contacting the swab tip must be avoided. The swab must be firmly scraped against the inside of each cheek between 15-20 times.

Incomplete buffer preparation.

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 solution should be stored at -20ºC. Do not freeze and thaw the solution more than 3 times.

2. 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.

3. 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.

Incorrect DNA elution step.

1. Ensure that Elution Buffer, TE or water is added into the **CENTER** of the GD 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.

2. Repeating the elution step will increase yield. Repeating the elution step using the eluate only will increase DNA concetration.

Eluted DNA Does Not Perform Well In Downstream Applications

Residual ethanol contamination.

Following the wash step, dry the GD Column with additional centrifugation at 14-16,000 x g for 5 minutes to ensure the GD Column membrane is completely dry.

Presto™ Buccal Swab gDNA Extraction Kit Test Data

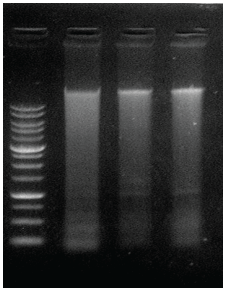


Figure 1. Genomic DNA from 3 individual cotton buccal swab samples was extracted using the Presto™ Buccal Swab gDNA Extraction Kit. The purified genomic DNA was analyzed by electrophoresis on a 0.8% agarose gel.
M = Geneaid 1 Kb DNA Ladder

Test	DNA Conc.	260/280	Yield
1	52.5 ng/μl	1.86	1.83 μg
2	34.8 ng/μl	1.90	1.22 μg
3	50.9 ng/μl	1.92	1.78 μg

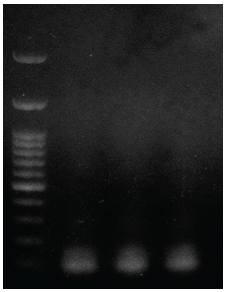


Figure 2. The purified genomic DNA was used as a template for amplifying partial human 5S ribosomal DNA (91 bp) by PCR. The PCR products were analyzed on a 0.8% agarose gel.
M = Geneaid 100 bp DNA Ladder

Related Genomic DNA Extraction Products

Genomic DNA Extraction and Purification		
Product	Package Size	Catalogue Number
Genomic DNA Mini Kit (Blood/Cultured Cell)	100/300 preps	GB100/300
Genomic DNA Midi Kit (Blood/Cultured Cell)	25 preps	GDI25
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	GPM10/25
Geneaid™ DNA Isolation Kit (Blood)	100/1,000 rxns	GEB100/01K(+)
Geneaid™ DNA Isolation Kit (Bacteria)	300/3,000 rxns	GEE300/03K(+)
Geneaid™ DNA Isolation Kit (Tissue)	150/1,500 rxns	GET150/1.5K(+)
Geneaid™ DNA Isolation Kit (Cultured Cell)	150/1,500 rxns	GEC150/1.5K(+)
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

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

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