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HiYield Genomic DNA Extraction Kit (Buccal Swab)

Cat. No.:	YGS100	YGS300
Product Name:	HiYield Genomic DNA Extraction Kit (Buccal Swab)	
Reactions:	100	300
Sample:	Buccal Swab	
Yield:	Up to 2 µg of pure genomic DNA per swab	
Elution Volume:	50-100 µl	
Format:	Spin Column	
Operation:	Centrifuge	
Operation Time:	Within 20 Minutes	

Description

HiYield Genomic DNA Extraction Kit (Buccal Swab) provides a fast and economical method for purification of total DNA (including genomic, mitochondrial and viral DNA) from buccal cells. The entire procedure can be completed 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 direct use in PCR or other enzymatic reactions. Furthermore, the purified DNA performs well in downstream analyses, such as quantitative PCR and STR analysis.

Features

1. Reliable purification of high-quality genomic DNA within 20 minutes.
2. Consistent and high yields, with an average DNA yield of 2 µg per buccal swab.
3. Complete removal of all contaminants for sensitive downstream applications.
4. No phenol, chloroform or alcohol.

Applications

Purified DNA is ready for direct use in PCR, Southern Blotting, Real-Time PCR, AFLP, RFLP, PADP.

Quality Control

The quality of HiYield Genomic DNA Extraction Kit (Buccal Swab) is tested on a lot-to-lot basis by isolation of genomic DNA from buccal cells. The purified DNA is quantified with a spectrophotometer and the yield of genomic DNA is more than 2 µg with A260/A280 ratio 1.8 - 2.0. The purified DNA is checked by electrophoresis.

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Contents

Item	YGS100	YGS300
GS1 Powder*	1 mg	1 mg
GS2 Buffer	60 ml	165 ml
GS3 Buffer	60 ml	165 ml
Proteinase K**	22 mg	65 mg
W1 Buffer	45 ml	130 ml
Wash Buffer (concentrated)***	25 ml	50 ml
Elution Buffer	30 ml	75 ml
Filter Column	100 pcs	300 pcs
GD Column	100 pcs	300 pcs
2 ml Collection Tube	300 pcs	900 pcs

* Add 1 ml of Elution Buffer to GS1 Powder then vortex to ensure GS1 Powder is completely dissolved to obtain a working solution of 1 µg/µl. Check the box on the bottle label showing Elution Buffer is added. Once GS1 Powder is dissolved completely, centrifuge for a few seconds to spin down the mixture. Divide the GS1 Buffer into convenient volumes in several RNase-free 1.5 ml microcentrifuge tubes. The GS1 Buffer should be stored at -20ºC. Do not freeze and thaw GS1 Buffer more than 3 times.

**Add ddH_20 (pH7.0-8.5) to Proteinase K (see the bottle label for volume) then vortex to ensure Proteinase K is completely dissolved. Check the box on the bottle showing ddH_20 is added. Once it is dissolved completely, centrifuge for a few seconds to spin down the mixture. For extended periods, store the ddH_20 and Proteinase K mixture at 4ºC. Use only fresh ddH_20 as ambient CO_2 can quickly cause acidification.

*** 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 showing absolute ethanol is added. Be sure and close the bottle tightly after each use to avoid ethanol evaporation.

Storage

HiYield Genomic DNA Extraction Kit (Buccal Swab) shall be shipped and stored dry at room temperature (15-25℃). With proper storage, HiYield Genomic DNA Extraction Kit (Buccal Swab) can be stored for up to 12 months without showing any deduction in performance and quality. For extended periods, store Proteinase K at 4ºC or -20 ºC.

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Buccal Swab Protocol

Please read the entire instruction manual prior to starting.

Things to Do before Starting:

1. Add 1 ml of Elution Buffer to GS1 Powder then vortex to ensure GS1 Powder is completely dissolved to obtain a working solution of 1 µg/µl. Check the box on the bottle label showing Elution Buffer is added. Once GS1 Powder is dissolved completely, centrifuge for a few seconds to spin down the mixture. Divide the GS1 Buffer into convenient volumes in several RNase-free 1.5 ml microcentrifuge tubes. The GS1 Buffer should be stored at -20ºC. Do not freeze and thaw GS1 Buffer more than 3 times.
2. Add ddH_20 (pH7.0-8.5) to Proteinase K (see the bottle label for volume) then vortex to ensure Proteinase K is completely dissolved. Check the box on the bottle showing ddH_20 is added. Once it is dissolved completely, centrifuge for a few seconds to spin down the mixture. For extended periods, store the ddH_20 and Proteinase K mixture at 4ºC. Use only fresh ddH_20 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 showing absolute ethanol is added. Be sure and close the bottle tightly after each use to avoid ethanol evaporation.

Additional Requirements:

Buccal Swabs, RNase-free 1.5 ml microcentrifuge tubes.

Caution: During operation, always wear a lab coat, disposable gloves and protective goggles.

Step 1 Buffer Preparation	1. Make sure GS1 Buffer, Proteinase K mixture and Wash Buffer are prepared according to the above instructions. 2. Transfer 1 µl of GS1 Buffer and 500 µl of GS2 Buffer per sample to a RNase-free 1.5 ml microcentrifuge tube and vortex shortly to mix. The mixture, GS12 Buffer, is for use in the Cell Lysis step.
Step 2 Sample Collection	To collect a sample, firmly scrape the swab against the inside of each cheek 20 times. Be 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. Ensure that the person providing the sample has not consumed any food 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.

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Step 3 Sample Preparation	1. Place the swab tip in a 1.5 ml microcentrifuge tube and remove it by either cutting or ejecting. 2. Add 500 µl of GS3 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 to lyse the sample. 3. 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 14,000-16,000 x g for 2 minutes to collect the remaining sample from the swab. 4. 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.
Step 4 Cell Lysis	1. Add 500 µl of GS12 Buffer (make sure 1 µl of GS1 Buffer was added) and then vortex IMMEDIATELY. 2. Incubate at 60ºC for 10 minutes. Vortex briefly every 5 minutes. It is essential that the sample and GS12 Buffer are mixed thoroughly to yield a homogeneous solution. Inverting the sample occasionally during incubation will facilitate Proteinase K digestion and cell lysis. Incubating with a shaking incubator is another convenient option. 3. 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 DNA Elution).
Step 5 DNA Binding	1. Add 500 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. 2. 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 14,000-16,000 x g for 1 minute then discard the flow-through. 3. Transfer the remaining lysate mixture to the GD Column. Centrifuge at 14,000-16,000 xg 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.

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Step 6 Wash	1. Add 400 µl of W1 Buffer to the GD Column. Centrifuge at 14,000-16,000 x g for 30 seconds then discard the flow-through. Place the GD Column back in the 2 ml Collection Tube. 2. Add 600 µl of Wash Buffer (absolute ethanol added) to the GD Column. Centrifuge at 14,000-16,000 x g for 30 seconds then discard the flow-through. Place the GD Column back in the 2 ml Collection Tube. 3. Centrifuge again for 3 minutes at 14,000-16,000 x g to dry the column matrix. NOTE: Additional centrifugation at 14,000-16,000 x g for 5 minutes or incubation at 60ºC for 5 minutes will completely dry the GD Column to avoid any residual ethanol carryover and ensure the most effective downstream applications.
Step 7 DNA Elution	1. Transfer the dried GD Column to a clean 1.5 ml microcentrifuge tube. 2. Add 100 µl[1] of pre-heated Elution Buffer[2], TE Buffer[3] or water[4] 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. 3. Centrifuge at 14,000-16,000 x g for 1 minute to elute the purified DNA. NOTE: [1] Standard elution volume is 100 µl. To increase DNA concentration, reduce the elution volume to 50-100 µl. To increase DNA recovery, repeat the DNA Elution step. [2] 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. [3]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. [4]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. DNA Eluted in water should be stored at -20ºC to avoid degradation.

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Troubleshooting

Problem	Possible Reasons / Solution
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** Add 1 ml of Elution Buffer to GS1 Powder then vortex to ensure GS1 Powder is completely dissolved to obtain a working solution of 1 µg/µl. Check the box on the bottle label showing Elution Buffer is added. Once GS1 Powder is dissolved completely, centrifuge for a few seconds to spin down the mixture. Divide the GS1 Buffer into convenient volumes in several RNase-free 1.5 ml microcentrifuge tubes. The GS1 Buffer should be stored at -20ºC. Do not freeze and thaw GS1 Buffer more than 3 times. Add ddH_20 (pH7.0-8.5) to Proteinase K (see the bottle label for volume) then vortex to ensure Proteinase K is completely dissolved. Check the box on the bottle showing ddH_20 is added. Once it is dissolved completely, centrifuge for a few seconds to spin down the mixture. For extended periods, store the ddH_20 and Proteinase K mixture at 4ºC. Use only fresh ddH_20 as ambient CO_2 can quickly cause acidification. 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 showing absolute ethanol is added. Be sure and close the bottle tightly after each use to avoid ethanol evaporation. **Incorrect DNA elution step** 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.
Eluted DNA Does Not Perform Well In Downstream Applications	**Residual Ethanol Contamination** Following the wash step, dry the GD Column with additional centrifugation at 14,000-16,000 x g for 5 minutes to ensure the GD Column membrane is completely dry.

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