Ver: 1907

Saliva gDNA Isolation Kit (Column)

（BW-GD2318）

Contents

Kit Contents 2

Introduction 2

Storage and Stability 3

Before Starting 3

Important Notes 3

Materials not Supplied 3

Safety Information 3

Protocol 4

Trouble Shooting Guide 6

Limited Use and Warranty 8

Kit Contents

Catalog#	BW-GD2318-00	BW-GD2318-01	BW-GD2318-02
Preps	10	50	250
DNA Micro Columns	10	50	250
2 mL Collection Tubes	10	50	250
Buffer BL	2.4 mL	12 mL	60 mL
DNA Wash Buffer*	3 mL	15 mL	3 x 24 mL
Elution Buffer	1.2 mL	6 mL	30 mL
Proteinase K	240 µL	1.2 mL	6 mL
User Manual	1	1	1

*Add 12 mL (BW-GD2318-00) or 60 mL (BW-GD2318-01) or 96 mL (BW-GD2318-02) 96-100% ethanol to each DNA Wash Buffer bottle before use.

Introduction

BEIWO Saliva gDNA Isolation Kit provides a fast and simple procedure for isolating genomic DNA from saliva samples collected. Purification is based on spin column chromatography. Human genomic DNA extracted from buccal epithelial cells and white blood cells found in saliva can be used in various applications in diagnostics. The isolated DNA can be used for the detection of biomarkers to diagnose a disease, follow the diseases progress or monitor the effects of a particular treatment. Saliva DNA can also be used to diagnose particular types of infections. Isolation of DNA from saliva has become an attractive alternative to isolation from blood or tissue due to the fact that sample collection is non-invasive, the samples can be collected by individuals with little training, and no special equipment is required. Saliva gDNA purified using BEIWO kit is of the highest quality, and is compatible with a number of downstream research applications including PCR, Southern Blot analysis, sequencing and microarray analysis.

Storage and Stability

Store Proteinase K at 4℃ upon receiving. All other materials can be stored at room temperature (15-25℃). The guaranteed shelf life is 12 months from the date of production.

Before Starting

Prepare all components and get all necessary materials ready by examining this user manual and become familiar with each step and pay special attention to the followings.

Important Notes

✪ Add 12 mL (BW-MGD2318-00) or 60 mL (BW-MGD2318-01) or 96 mL (BW-MGD2318-02) 96-100% ethanol to each DNA Wash Buffer bottle before use.

✪ Prior to collection of saliva samples, the donor should rinse their mouth with a few milliliters of water for 10 seconds in order to remove any food particles that may be present. If food particles are present they may cause clogging of the column. 10 minutes after rinsing, collect saliva by spitting into a sterile collection tube or vital (not provided). The amount of saliva collected should be at least 100 µL but not more than 2 mL.

✪ Ensure the availability of centrifuge capable of 12,000 rpm.

✪ *Carry out all centrifugations at room temperature.*

Materials not Supplied

✪ Sterile collection tubes and sterile microfuge tubes.

✪ Water bath (55℃).

✪ Isopropanol.

Safety Information

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, please consult the appropriate material safety data sheets.

Protocol

1. Sample pretreatment:

a. Fresh saliva sample

Collect 250 µL fresh saliva sample into a sterile 2 mL centrifuge tube and add 250 µL of Saliva Preservative.

Note: The Saliva Preservative needs to be purchased separately.

b. Saliva sample with protective solution

Add 400-500 µL saliva sample containing the protective solution (Saliva Preservative : Saliva = 1:1) into a sterile 2 mL centrifuge tube.

c. Oral swab sample

Add Saliva Preservative and PBS to 2 mL centrifuge tube (Saliva Preservative : PBS = 1:1), mix well, and the swab sample was cut and completely immersed in the liquid.

Note: Because the cotton swab absorbs liquid, make sure that the mixture has at least 400 µL.

d. Oral swab sample with protective solution

Proceed directly to step **2**.

2. Add **20 µL Proteinase K** (vortex before use). Mix by vortexing and incubate at 55℃ for 10-15 minutes.

3. Add **200 µL Buffer BL** to the saliva sample. Mix by vortexing and incubate at 55℃ for 5 minutes.

4. Add 720 µL Isopropanol and mix by vortexing for a few seconds.

5. Assemble a **DNA Micro Column** with a **2 mL Collection Tube**.

6. Apply up to 750 µL of the lysate to the **DNA Micro Column** and centrifuge for 1 minute at 6,000 rpm.

7. Discard the flow-through and reassemble the **DNA Micro Column** to the **2 mL Collection Tube**.

8. Repeat step **7**, until all the lysate has passed through the **DNA Micro Column**.

9. Apply **500 µL DNA Wash Buffer** (*Add ethanol to DNA Wash Buffer before use*) to the **DNA Micro Column** and centrifuge for 1 minute at 8,000 rpm. Discard the flow-through and reassemble the **DNA Micro Column** to the **2 mL Collection Tube**.

10. Apply **500 µL DNA Wash Buffer** to the **DNA Micro Column** and centrifuge for 1 minute at maximum speed. Discard the flow-through and reassemble the **DNA Micro Column** to the **2 mL Collection Tube**.

11. Spin the **DNA Micro Column** for 2 minutes at maximum speed in order to thoroughly dry the column. Discard the **2 mL Collection Tube**.

12. Place the **DNA Micro Column** into a sterile 1.5 mL microfuge tube, add **50-100 µL Elution Buffer** or ddH_2O to the column. Incubate for 5 minutes at 55℃.

13. Centrifuge for 2 minutes at 2,000 rpm, followed by a second spin for 1 minute at maximum speed.

14. The purified DNA sample may be stored at 4℃ for a few days. It is recommended that samples be placed at -20℃ for long-term storage.

Trouble Shooting Guide

Problems	Possible Reasons	Suggested Improvements
The DNA Micro Column is clogged	Centrifugation speed was too low or spin time was inadequate	Check the centrifuge to ensure that it is capable of generating the required RPMs. Sufficient centrifugal force is required to move the liquid phase through the column. Also ensure that the correct spin times are followed. Spinning for a few additional minutes will help.
	The sample is too large	Too many cells were applied to the column. Ensure that no more than 0.5 mL of preserved saliva is applied to the column. Clogging can be alleviated by centrifuging for a longer period of time until the lysate passes through the column.
	The lysate/binding solution mixture is not homogeneous	To ensure a homogeneous solution, vortex for 10-15 seconds before applying the lysate to the micro column.
The yield of genomic DNA is low	Incomplete lysis of cells	Increased Proteinase K incubation time at 55°C may result in increased yields
	The DNA elution is incomplete	Perform an additional centrifugation of 2 minutes at 14,000 rpm to ensure that all the DNA is eluted.
	DNA concentration in the saliva sample being used is low	Some saliva samples contain very little DNA. This varies from individual to individual based on numerous variables. Increased proteinase K incubation time at 55°C may result in increased yields.

DNA does not perform well in downstream applications	DNA was not washed with DNA Wash Buffer	Traces of salt from the binding step may remain in the sample if the column is not wash with DNA Wash Buffer. Salt may interfere with downstream applications, and thus must be washed from column.
	Ethanol carryover	Ensure that the dry spin after the column wash steps is performed, in order to remove traces of ethanol prior to elution. Ethanol is known to interfere with many downstream applications.
RNA is present in eluted DNA	RNA is coeluted with the DNA	Carry out a digestion with RNase A on the elution if the RNase present will interfere with downstream applications. Refer to manufacturer's instructions regarding amount of enzyme to use, optimal incubation time and temperature.

Limited Use and Warranty

This product is intended for *in vitro* research use only. Not for use in human.

This product is warranted to perform as described in its labeling and in BEIWO's literature when used in accordance with instructions. No other warranties of any kind expressed or implied, including, without limitation, implied warranties of merchantability or fitness for a particular purpose, are provided by BEIWO. BEIWO's sole obligation and purchaser's exclusive remedy for breach of this warranty shall be, at the option of BEIWO, to replace the products, BEIWO shall have no liability for any direct, indirect, consequential, or incidental damage arising out of the use, the results of use, or the inability to use it product.

For technical support or learn more product information, please contact us or visit our website.

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