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User's Manual and Instructions

Saliva DNA Isolation Kit

Catalog Number: K5011050

Introduction

The Saliva DNA Isolation Kit is designed for the purification of genomic DNA and circulating DNA from freshly collected saliva samples. Purification is performed with the use of Spin columns which bind the DNA. No phenol-chloroform and precipitation steps are involved in this procedure. Isolation of the DNA is performed by lysing the cells in a solution containing chaotropic salts and detergent. To assist in lysing the cells, proteinase K is also added to the mixture. Isopropanol is added to the samples, before applying to the spin columns. Under these conditions the DNA binds to the membrane while other contaminants are washed through. The columns are then washed to further remove protein, buffer components and other contaminants using two ethanol-containing wash buffers and the final genomic DNA product is eluted in TE. The final DNA product can be used directly for quantitative PCR and other downstream applications.

Feature

- No phenol-chloroform
- No precipitation
- Total isolation time: <25 min.
- Sample range: 50 µl to 200 µl
- DNA yield: 0.25-4 µg/200 µl fresh saliva

Kit Contents

Item	Part #	Amount	Storage
1. Lysis Binding Buffer C	K5011050-1	30 ml	RT
2. Wash Buffer 1	K5011050-2	14 ml	RT
3. Wash Buffer 2	K5011050-3	7 ml	RT
4. TE Buffer	K5011050-4	10 ml	RT
5. Spin column set	K5011050-5	50 units	RT
6. Proteinase K Solution	K5011050-6	250 µl	-20°C

Special Handling instructions:

- Lysis Binding Buffer C contains hexadecyltrimethylammonium bromide and guanidine hydrochloride. Care should be taken in handling and disposal of samples.
- Wash Buffer 1 contains guanidine hydrochloride as a component. Care should be taken in handling and disposal of samples.

Storage Conditions

All of contents of the Saliva and Septum DNA Isolation Kit except the Proteinase K Solution should be stored at room temperature. The Proteinase K Solution should be stored at -20°C. The kit is stable for one year under these conditions.

Technical Assistance

Please refer any technical questions to TechSupport@biochain.com.

Sample Size and Type

Before collection of the saliva or septum sample, have the donor rinse their mouth with water to remove any excess food particles. The collected sample should be stored at <4°C if being processed within 4

hours and at -80°C if being processed at a later date or time. The isolation procedure was designed for purification from 200 µl of sample but may be used to isolate DNA from up to 600 µl of saliva sample. If increasing the sample volume from 200 µl, adjust the amount of Lysis Binding Buffer C, Proteinase K Solution, and Isopropanol proportionately.

Buffer Concentrates
Wash buffers 1 and 2 are provided as concentrates that require the addition of 100% ethanol to them before use.

Reagents and Equipment to be Supplied by the User
• Pipetteman (multichannel pipettors desirable)
• 1.5 ml tubes
• Disposable gloves
• 100% ethanol
• Isopropanol
• Distilled deionized water
• A heat block or incubator set between 50°C-57°C.
• A table-top centrifuge capable of providing >13k rpm rotor.

Before starting:

- Add 14 ml of 100% ethanol to Wash Buffer 1 and mark the bottle to indicate the addition of ethanol.
- Add 28 ml of 100% ethanol to Wash Buffer 2 and mark the bottle to indicate the addition of ethanol.

Protocol:

1. Transfer (up to) 200 µl of saliva or septum sample to a 1.5 ml tube.
2. Add 600 µl of **Lysis Binding Buffer C** to the sample and mix well by vortexing briefly. If necessary vortex until sample and lysis buffer are evenly mixed.
3. Add 5 µl for the Proteinase K Solution and briefly mix by vortexing.
4. Allow the mixture to incubate at 50°C-57°C for 10 minutes.
5. After the incubation period, add 400 µl of isopropanol and mix well.
6. Apply 600 µl of the sample to a Spin column and centrifuge at full speed for 1 min.
7. Discard the flowthrough from the collection tube and reapply the Spin column onto the collection tube. Apply the remaining 600 µl of sample and centrifuge at full speed for 1 min. If processing more than 200 µl of saliva, repeat step 5 until all of the sample has been passed through the column.
8. Discard the flowthrough from the collection tube and reapply the Spin column onto the collection tube.
9. Add 500 µl of Wash Buffer 1 to the Spin column and centrifuge at full speed for 1 min. Ensure that ethanol has been added to Wash Buffer 1.
10. Discard the flowthrough. Add 600 µl of Wash Buffer 2 to the Spin column and centrifuge at full speed for 1 min. Ensure that ethanol has been added to Wash Buffer 2.
11. Discard the flowthrough and reapply the Spin column onto the collection tube. Dry the Spin column by centrifuging at full speed for 1 min.
12. Place the Spin column onto a new 1.5ml microcentrifuge tube for elution. Apply 30-100 µl of TE Buffer to the center of the Spin column membrane. A lower volume of TE elution buffer will lead to a higher concentration of DNA. Using a higher volume of TE will result in higher recovery of DNA from the column.

Kit Performance

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Table 1 shows the technical specifications of the DNA isolation kit.

TECHNICAL SPECIFICATIONS	
Yield	0.25-4 µg/200 µl fresh saliva
Purity	$UV_{260/280}$> 1.8
DNA size	99% of DNA is >20kb
Total time of prep	less than 25 min

100 ng of DNA isolated from this kit was run on a 1% agarose gel.