User's Manual

Product: Stool DNA Isolation Kit

Catalog Number: K5011250

Introduction

The Stool DNA Isolation Kit is designed for the purification of genomic DNA fragment from stool samples in a spin column format. No phenol-chloroform, protease or precipitation steps are involved in isolating DNA.

Isolation of the DNA is performed by lysing the cells in a solution containing chaotropic salts and detergent. 100% ethanol is added to the sample, before applying to the spin columns. Under these conditions the DNA binds to the membrane while other contaminants are washed through. The column is then washed to further remove protein, buffer components and other contaminants using two ethanol-containing wash buffers. Incubation of the Spin column membrane with TE buffer and a final centrifugation step leads to the elution of the DNA. The final DNA product can be used directly for quantitative PCR and other downstream applications.

Feature

- No phenol-chloroform
- No precipitation
- No protease
- Total <30 min.
- Sample range: 200 mg Stool

Kit Contents

Item	Part #	Amount	Storage
1. Lysis Binding Buffer PC	K5011250-1	55 ml	RT
2. Wash Buffer WBR1	K5011250-2	14 ml	RT
3. Wash Buffer 2	K5011250-3	16 ml	RT
4. TE Buffer	K5011250-4	10 ml	RT
5. Spin column set	K5011250-5	50 units	RT

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 contain guanidine hydrochloride as a component. Care should be taken in handling and disposal of samples.

Storage Conditions

All of the components of the Stool DNA Isolation Kit should be stored at room temperature. The kit is stable for one year under these conditions.

Technical Assistance

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

Buffer Concentrates

Wash buffers WBR1 and Wash Buffer 2 require the addition of 100% ethanol before use.

Reagents and Equipment to be Supplied by the User

• Pipetteman (multichannel pipettors desirable)
• 1.5 ml microcentrifuge tubes
• 2.0 ml microcentrifuge tubes
• Disposable gloves
• 100% ethanol
• 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 16 ml of 100% ethanol to Wash Buffer 2 and mark the bottle to indicate the addition of ethanol.

Protocol:

1. Transfer (up to) 200 mg of Stool sample to a 2 ml tube.
2. Add 1 ml of **Lysis Binding Buffer PC** to the sample and mix well by vortexing for 1 min.
3. Allow the mixture to incubate at room temperature for 10 minutes.
4. After incubation, vortex tube for 1 min and then centrifuge at full speed for 5 min.
5. Remove 800 µl of the supernatant and transfer to a new 1.5 ml tube. Discard the remaining sample and pellet.
6. Add 400 µl of **ethanol** to the supernatant and mix well.
7. Apply 600 µl of the sample to a Spin column and centrifuge at full speed for 1 min.
8. 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.
9. Discard the flowthrough from the collection tube and reapply the Spin column onto the collection tube.
10. Add 500 µl of **Wash Buffer WBR1** to the Spin column and cap the tube. Incubate the Spin column at room temperature for 1 min. After incubation, centrifuge at full speed for 1 min. Ensure that ethanol has been added to Wash Buffer WBR1.
11. 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.
12. Discard the flowthrough and reapply the Spin column onto the collection tube. Dry the Spin column by centrifuging at full speed for 1 min.
13. Place the Spin column onto a new 1.5ml microcentrifuge tube for elution. Apply 100 µl of TE Buffer to the center of the Spin column membrane. Cap the spin column and incubate for 1-5min at room temperature. Centrifuge the Spin column at full speed for 1 min to elute the DNA. An additional elution may be performed by repeating this step a second time. Incubating the membrane with TE buffer up to 5 mins helps recover more DNA. 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

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

TECHNICAL SPECIFICATIONS	
Yield	>8 µg/200 mg Stool
Purity	$UV_{260/280}$> 1.8
DNA size	99% of DNA is >20kb
Total time of prep	less than 30 min

DNA isolated from Stool using this kit was run on a 1% agarose gel.