BIOTECON Diagnostics

foodproof® Sample Preparation Kit I

Order No. S 400 04

Quick Reference Procedure

Version 2, March 2012

A. Kit Content

All solutions except **food**proof Sample Preparation Kit I Lysis Buffer (vial 1) are clear, and should not be used when precipitates have formed. If precipitates have formed, warm the solutions at 15 – 25 °C or in a 37 °C water bath until the precipitates have dissolved. Store all reagents at 15 to 25 °C.

B. Chemical Hazard

The **food**proof Sample Preparation Kit I Binding Buffer (vial 2) contains irritating compounds that are harmful when brought in contact with skin, inhaled, or swallowed. Always store and use this buffer away from food for humans and animals. Always wear gloves, and follow standard safety precautions during handling.

C. Number of Preparations

100 isolations

D. Storage and Stability

• Store the kit at 15 °C to 25 °C through the expiration date printed on the label.
• Once the kit is opened, store the kit components as described in the following Kit Contents table:
Note: Inappropriate storage at 2–8°C (refrigerator) or -15 to -25°C (freezer) will adversely impact nucleic acid purification when precipitates form in the solutions.

Vial / Cap Color	Label	Contents / Function
1 red cap	**food**proof Sample Preparation Kit I Lysis Buffer	• 20 ml • For lysis of cells and extraction of DNA.
2 green cap	**food**proof Sample Preparation Kit I Binding Buffer	• 30 ml • For binding of DNA to glass fiber fleece.
3 blue cap	**food**proof Sample Preparation Kit I Wash Buffer	• 20 ml, add 80 ml absolute ethanol. • For removing impurities.
4 colorless cap	**food**proof Sample Preparation Kit I Elution Buffer	• 40 ml • For elution of DNA.
5	**food**proof Sample Preparation Kit I Filter Tubes	• 2 bags with 50 polypropylene tubes with two layers of glass fiber fleece. • For use with up to 700 µl sample volume.
6	Collection Tubes	• 2 bags with 50 polypropylene tubes (2 ml).

E. Additional Equipment and Reagents Required

• Ethanol, absolute
• Standard tabletop microcentrifuge capable of a 13,000 × g centrifugal force (*e.g.,* Eppendorf 5415C or equivalent)
• Microcentrifuge tubes, 1.5 ml, sterile
• Heating Unit

F. Before you Begin

In addition to the ready-to-use solutions supplied with the kit, you will need the following working solutions; preparation of working solutions is required:

Bottle / Cap Color	Content	Preparation of working solution	Storage and stability
3 blue	**food**proof Sample Preparation Kit I Wash Buffer	Add 80 ml absolute ethanol to **food**proof Sample Preparation I Wash Buffer. Note: Label and date bottle after ethanol is added.	Store at 15 – 25 °C. Stable until the expiration date printed on kit label.

G. Procedure

The following protocol describes the DNA isolation from 1 ml enrichment culture.

Step	Action	Volume	Time/g /Time/Temp.
1	• Shake the enrichment culture gently and let settle. • Transfer the sample (supernatant) to a 1.5 ml reaction tube.	1 ml	5 – 10 min
2	Centrifuge		5 min at 8,000 × g
3	• Remove the supernatant with a pipette, discard and inactivate appropriately. • Resuspend the pellet in **food**proof Sample Preparation Kit I Lysis Buffer (bottle 1, red cap), mixing the **food**proof Sample Preparation Kit I Lysis Buffer well while pipetting. • Incubate Note: Ensure that the reaction tube is firmly shut. • Mix the lysate by vortexing. • Centrifuge	200 µl	 95 °C for 10 min 2 s 1 min at 12,000 × g
4	• Add **food**proof Sample Preparation Kit I Binding Buffer (bottle 2, green cap), to a new 1.5 ml reaction tube.	300 µl	
5	• Transfer the entire supernatant (from step 3) to the reaction tube with the **food**proof Sample Preparation Kit I Binding Buffer (step 4). • Mix well by pipetting up and down.	approx. 200 µl	
6	• Pipet the entire mixture into the upper reservoir of a combined Sample Preparation Filter Tube-Collection Tube assembly.	approx. 500 µl	
7	Centrifuge		1 min at 5,000 × g
8	• Discard the flow-through. • Add **food**proof Sample Preparation Kit I Wash Buffer working solution (bottle 3, blue cap) to the upper reservoir. • Centrifuge	450 µl	 1 min at 5,000 × g
9	Repeat step 8.		
10	• Discard the flow-through. • Centrifuge to remove residual **food**proof Sample Preparation Kit I Wash Buffer.		10 s at max. speed (13,000 × g)
11	• Insert filter tube in a clean 1.5 ml reaction tube. • Add pre-warmed (70 °C) **food**proof Sample Preparation Kit I Elution Buffer (bottle 4, colorless cap) onto the glass fiber fleece. • Incubate	50 µl	 15 – 25 °C for 1 – 2 min
12	Centrifuge Result: The reaction tube now contains the eluted DNA.		1 min at 5,000 × g

Note: For further information please refer to: www.bc-diagnostics.com/?cid=1195722740&lang=1

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