foodproof® Sample Preparation Kit II

Order No. S 400 05

Quick Reference Procedure

Version 2, March 2012

A. Number of Preparations

100 isolations

B. 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 II Lysis Buffer	• 20 ml • For lysis of cells and extraction of DNA.
2 green cap	**food**proof Sample Preparation Kit II Binding Buffer	• 20 ml • For binding of DNA to glass fiber fleece.
3 blue cap	**food**proof Sample Preparation Kit II Wash Buffer	• 20 ml, add 80 ml absolute ethanol. • For removing impurities.
4 colorless cap	**food**proof Sample Preparation Kit II Elution Buffer	• 40 ml • For elution of DNA.
5 purple cap	**food**proof Sample Preparation Kit II Proteinase K	• Lyophilizate • 100 mg • For protein digestion and inactivation of endogenous nucleases.
6 white cap	**food**proof Sample Preparation Kit II Lysozyme	• Crystalline • 11 mg, for digestion of bacterial cell wall.
7	**food**proof Sample Preparation Kit II[1)] Filter Tubes	• 2 bags with 50 polypropylene tubes with two layers of glass fiber fleece • For use with up to 700 µl sample volume.
8	Collection Tubes	• 2 bags with 50 polypropylene tubes (2 ml).

C. Additional Equipment and Reagents Required

• Ethanol, absolute
• Isopropanol, absolute
• Water, double-distilled
• 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

D. 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 cap	**food**proof Sample Preparation Kit II Wash Buffer	Add 80 ml absolute ethanol to **food**proof Sample Preparation Kit II 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.
5 purple cap	**food**proof Sample Preparation Kit II Proteinase K	Dissolve **food**proof Sample Preparation Kit II Proteinase K in 5 ml double-distilled water; aliquot solution.	Store at -15 to -25 °C, Stable for 12 months.
6 white cap	**food**proof Sample Preparation Kit II Lysozyme	Dissolve **food**proof Sample Preparation Kit II Lysozyme in 1.1 ml double- distilled water; aliquot solution.	Store at -15 to -25 °C, Stable for 12 months.

E. Caution
In order to avoid cross-contamination, use sterile disposable polypropylene tubes and filter tips. Always wear gloves during the assay, and follow safety precautions to minimize contact when handling. Follow all universal safety precautions governing work with biohazardous materials (*e.g.*, wear lab coats at all times). Also, properly dispose of all contaminated materials, decontaminate work surfaces, and use a biosafety cabinet whenever aerosols might be generated.

F. Additional reagents required
Isopropanol, absolute

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 II Lysis Buffer (bottle 1, red cap), Mix the **food**proof Sample Preparation Kit II Lysis Buffer well. • Add **food**proof Sample Preparation Kit II Lysozyme working solution (see section 3.2.), then mix gently but thoroughly. • Incubate	200 µl 10 µl	 37 °C for 10 min
4	• Add **food**proof Sample Preparation Kit II Binding Buffer (bottle 2, green cap), then mix gently but thoroughly by pipetting up and down. • Add **food**proof Sample Preparation Kit II Proteinase K working solution (see section 3.2.). • Mix gently but thoroughly by pipetting up and down. • Incubate	200 µl 40 µl	 72 °C for 10 min
5	• Add Isopropanol • Mix well	100 µl	
6	Centrifuge		15 s at 12,000 × g
7	• Pipet the entire supernatant into the upper reservoir of a combined **food**proof Sample Preparation Filter Tube-Collection tube assembly.	approx. 550 µl	
8	Centrifuge		1 min at 5,000 × g
9	• Discard the flow-through. • Add **food**proof Sample Preparation Kit II Wash Buffer working solution (bottle 3, blue cap) to the upper reservoir. • Centrifuge	450 µl	 1 min at 5,000 × g
10	Repeat step 9.		
11	• Discard the flow-through. • Centrifuge to remove residual **food**proof Sample Preparation Kit II Wash Buffer.		10 s at max. speed (13,000 × g)
12	• Insert filter tube in a clean 1.5 ml reaction tube. • Add pre-warmed (70 °C) **food**proof Sample Preparation Kit II Elution Buffer (bottle 4, colorless cap) onto the glass fiber fleece. • Incubate	50 µl	 15 – 25 °C for 1 – 2 min
13	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=1195722828&lang=1

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