For food testing purposes
FOR *IN VITRO* USE ONLY

foodproof® **Sample Preparation Kit I**

Version 1, October 2007

For isolation of bacterial DNA from enrichment cultures of food samples prior to PCR analysis

Order No. S 400 04

Kit for 100 Isolations

Store the kit at 15 to 25 °C

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1. What this Product Does

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.

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.

Number of Preparations

100 isolations

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).

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

2. How to Use this Product

2.1 Product overview

Test Principle

Following concentration by centrifugation, the cells are lyzed during a short incubation with the provided **food**proof Sample Preparation Kit I Lysis Buffer. After clearing of the lysis mixture by centrifugation, the DNA selectively binds to special glass fibers pre-packed in the Filter Tube. Bound DNA is purified in two “wash-and-spin” steps to remove potential PCR inhibitors, then a low-salt elution releases the DNA from the glass fiber. This simple method eliminates the need for organic-solvent extractions and DNA precipitation, thus providing rapid, simultaneous purification of many samples.

Basic steps

Stage	Description
1	Cells are lyzed by incubation with the **food**proof Sample Preparation Kit I Lysis Buffer; DNA is released.
2	Clearing of the mixture by centrifugation.
3	DNA is bound to the glass fibers pre-packed in the **food**proof Sample Preparation Filter Tube.
4	Washing of bound DNA to remove salts, proteins, and other cellular impurities.
5	Purified DNA is recovered using the **food**proof Sample Preparation Kit I Elution Buffer.

Application

The **food**proof Sample Preparation Kit I is optimized for isolation of bacterial DNA from enrichment cultures of various food samples (raw material and processed food). The quality of the DNA obtained with the kit is highly suitable for applications using any PCR System.

Sample Material

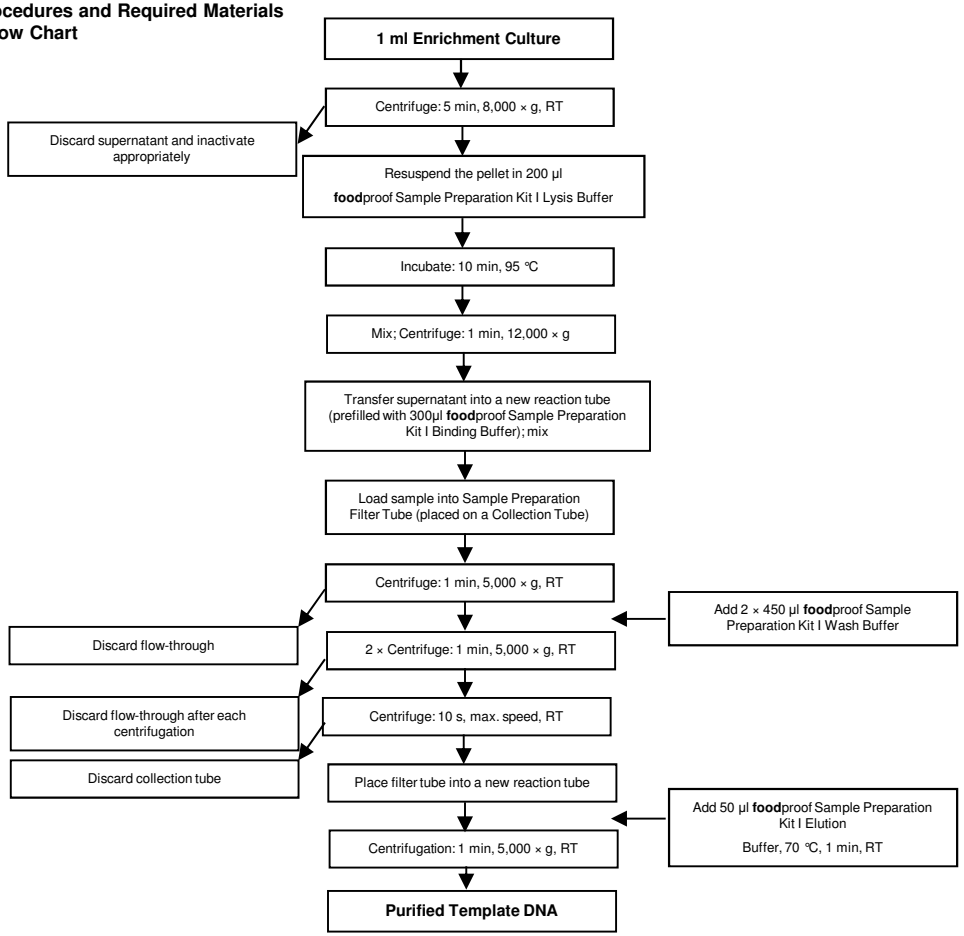
1 ml enrichment culture of food samples (raw material and processed food).

Quality Control

- Buffered peptone water spiked with about 10^3 cfu/ml *Salmonella Enteritidis* is extracted and purified as described below.
- 5 µl of the eluate is analyzed using the **food**proof *Salmonella* Detection Kit (Cat. No. R 300 27). As expected, the resulting amplification signal is obtained.
- The absence of contaminating DNA is controlled by an additional DNA preparation and a subsequent PCR test with unspiked peptone water as the sample material. As expected, no amplification product is obtained.

3. Procedures and Required Materials

3.1 Flow Chart



3.2 Before you Begin

Preparation of Kit Working Solutions

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

3.3 Isolation Procedure

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.

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	

Step	Action	Volume	Time/g /Time/Temp.
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

Storage of Samples

IF you want to…	THEN
Continue	Use the eluted DNA directly.
Stop	Store the DNA at -15 to -25 °C for later analysis.

4. Typical Results

Purity

Purified DNA is free of other cellular components and DNA polymerase inhibitors.

5. Appendix
5.1 Troubleshooting

Problem	Possible Cause	Recommendation
Low DNA yield or purity	Kit stored under non optimal conditions.	Store the kit at 15 – 25 °C at all times upon arrival.
	Buffers or other reagents were exposed to conditions that reduced their effectiveness.	• Store all buffers at 15 – 25 °C. • Close all reagent bottles tightly after each use to preserve pH and stability, and to prevent contamination.
	Ethanol not added to **food**proof Sample Preparation Kit I Wash Buffer.	• Add absolute ethanol to the Wash Buffer before using. • After adding ethanol, mix the **food**proof Sample Preparation Kit I Wash Buffer well, and store at 15 – 25 °C. • Always mark **food**proof Sample Preparation Kit I Wash Buffer bottle to indicate the addition of ethanol.
	Reagents and samples not completely mixed.	Always mix the sample tube well after addition of each reagent.
	Not enough target organisms in enrichment culture.	Prolong the incubation phase.
Absorbency (A_{260}) reading of product too high	Glass fibers which can coelute with DNA, scatter light.	• After elution step is complete, remove **food**proof Sample Preparation Kit I filter from tube containing eluted sample, and spin sample tube for 2 min at maximum speed. • Transfer supernatant into a new tube without disturbing the glass fibers at the bottom of the original tube.
Sample "pops" out of wells in agarose gels	Eluate containing the purified DNA product is contaminated with ethanol from the **food**proof Sample Preparation Kit I Wash Buffer.	• After the last wash step, ensure the flow-through containing Wash Buffer does not contact the bottom of the Filter Tube. • If this has occurred, empty collection tube, reinsert the contaminated filter tube, and re-centrifuge for 30 sec.

5.2 Helpful hints

Centrifugation

For convenience, the following table shows corresponding centrifugal forces (g) for selected rotations per minute (rpm) when working with a standard table top microcentrifuge (*e.g.,* such as Eppendorf 5415 C).

Rotations per minute (rpm)	Centrifugation force (g)
14,000	15,800
12,000	11,600
10,000	8,000
8,000	5,200
5,000	2,000
3,000	720
1,000	80

6. Supplementary Information

6.1 Ordering Information

BIOTECON Diagnostics is offering a broad range of reagents and services. For a complete overview and for more information, please visit our website at www.bc-diagnostics.com.

6.2 Trademarks

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6.3 Contact and Support

If you have questions or experience problems with this or any other product of BIOTECON Diagnostics, please contact our Technical Support staff (for details see www.bc-diagnostics.com). Our scientists commit themselves to providing rapid and effective help. We also want you to contact us if you have suggestions for enhancing our product performance or using our products in new or specialized ways. Such customer information has repeatedly proven invaluable to us and the worldwide research community.

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