

For food testing purposes FOR *IN VITRO* USE ONLY

foodproof® ShortPrep I

Version 1, October 2007

For preparation of PCR templates from enrichment cultures of food samples

Catalog No. S 400 01

Kit for 96 reactions

Store the kit at 15 to 25 ℃



NordVal Validation



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1. What this Product Does Storage and Stability

The **food**proof ShortPrep I Lysis Reagent is guaranteed to be stable until the expiration date printed on the label when stored at 15 to 25 °C.

Kit Contents

Component	Amount
Reaction tubes with 200 μl ready-to-use food proof ShortPrep I Lysis Reagent	96 tubes

Additional Equipment and Reagents Required

- Standard tabletop microcentrifuge capable of a 8000 x g centrifugal force (e.g., Eppendorf 5415 C or equivalent)
- · Heating Unit

Applicability Statement

The kit can be used to prepare DNA from up to 50 µl of bacterial enrichment cultures. The kit is optimized for preparation of food (raw materials and processed food) enrichment cultures. The quality of the DNA obtained with the kit is suitable for applications using any PCR instrument. The **food**proof ShortPrep I Kit is AOAC-RI and NordVal validated for use with a variety of foods (raw materials and processed foods). It has been AOAC-RI validated for use in **conjunction** with the **food**proof *Salmonella* Detection Kit. Moreover, the kit has been NordVal validated in combination with the **food**proof *Salmonella* Detection Kit with the ISO 6579 method for 5 food groups (meat, fish, milk, eggs and vegetable products) as well as for the matrix groups environmental tests (metal, glass, and plastic surfaces) and pet foods (cat food, dog food and fish food).

Preparation of the enrichment culture of some food material is not recommended with this kit. In particular, the DNA extracts from some raw materials of plant origin (e.g., spices) may still contain substances that interfere with the amplification or with the fluorescence signal detection. For such materials, preparation and purification with the **food**proof Sample Preparation Kit I is recommended.

2. How to Use this Product

Precautions

In order to avoid cross-contamination, use filter tips only. Follow all universal safety precautions governing work with biohazardous materials (*e.g.*, wear lab coats and gloves at all times). Properly dispose of all contaminated materials, decontaminate work surfaces, and use a biosafety cabinet whenever aerosols might be generated.

Before you Begin

- Warm the heating unit to 95-100 ℃.
- In order to collect the **food**proof ShortPrep I Lysis Reagent at the bottom of the tube, centrifuge the reaction tube with the ready-to-use **food**proof ShortPrep I Lysis Reagent at 500 × g for 30 60 s.



Enrichment Protocols

- Pre-enrichment according to FDA-BAM instructions: Bacteriological Analytical Manual, 8th Edition, Revision A, 1998, Chapter 5 (www.cfsan.fda.gov/~ebam/bam-5.html): Salmonella, Andrews H.W., Hammack S.T., 1999-DEC, 2000-MAR, and 2000-AUG Final revision on 2000-NOV-14. (Suitable for all food stuffs except for those mentioned below for which the USDA should be used. For environmental samples this kit has been validated with the ISO method.)
- Pre-enrichment according to USDA instructions: USDA Microbiology Laboratory Guidebook, Chapter 4: Isolation and Identification of Salmonella from Meat, Poultry and Egg Products, Sparling P., 3rd Edition/2004, www.fsis.usda.gov/PDF/MLG 4 03.pdf
- Pre-enrichment according to ISO instructions: ISO 6579, Microbiology of food and animal feeding stuffs Horizontal method for the detection of Salmonella - (German version: DIN EN ISO 6579-1: March 2003)

In case that other suitable enrichment procedures have been validated by the user these may be used instead.

Note: After pre-enrichment a sub-cultivation 1/10 in pre-warmed brain heart infusion broth (e.g. 1 ml sample + 9 ml broth) for 3 h at 37 °C is recommended.

Isolation Protocol

The following protocol describes the DNA isolation from 50 µl bacterial enrichment culture.

1	Shake the enrichment culture gently and let settle for 5 to 10 min.		
2	Transfer the sample (50 µl supernatant) to the reaction tube containing the ready-to-use food proof ShortPrep I Lysis Reagent. Mix by vortexing. Note: Ensure that the reaction tube is firmly closed.		
3	Incubate for 10 min in the heating unit at 95 - 100 ℃.		
4	Carefully remove the reaction tube from the heating unit, and allow the tube to sit for 1 min at 15 - 25 ℃.		
5	Mix by vortexing.		
6	Centrifuge for 1 min at 8,000 × g.		
7	The supernatant now contains the extracted DNA and can be used directly for PCR. Strictly avoid transferring fractions of the sediment to the PCR reaction, because this might cause PCR inhibition. The sample still contains proteins, RNA, and other compounds. Thus, long-term storage or archival storage of prepared DNA samples is not recommended. Alternatively, you may store the DNA at -15 to -25 °C for later analysis.		



3. Troubleshooting

Problem	Possible Cause	Recommendation
DNA sample inhibits PCR	Enrichment culture contains too many PCR inhibitors.	Perform a subcultivation (e.g., 1:10 dilution in fresh enrichment broth). Use the food proof Sample preparation Kit I for sample preparation.
	Some of the centrifugation pellet transferred to the PCR.	Use the top of the supernatant as PCR template. Always centrifuge the DNA sample before performing PCR.
Low DNA yield	Inappropriate storage of kit components.	Store the reaction tube with ready-to-use food proof Short Prep I Lysis Reagent at 15 to 25°C.
	Enrichment culture contains substances that reduce the DNA extraction efficiency.	Perform a subcultivation (e.g., 1:10 dilution in fresh enrichment broth).
	Not enough target organisms in enrichment culture.	Prolong the incubation phase.
Lid of the reaction tube opens during or after heating.	Reaction tube not firmly closed.	Ensure that all reaction tubes are firmly closed before heating. Use lid clips for closing the tubes. Use a heating unit that enables removal of the tube without contact with the tube.

4. Additional Information on this Product How this Product Works

The **food**proof ShortPrep I is designed for the rapid preparation of bacterial DNA for direct use in PCR. In less than 20 minutes, the kit generates PCR template DNA from 50 μ I of bacterial enrichment culture. The obtained DNA can be used directly in PCR using the LightCycler® Carousel-Based System. The kit includes a prefilled special Lysis Reagent that eliminates the need for hazardous organic extractions or chaotropic agents. The entire DNA preparation can be performed in a single tube, minimizing handling steps and exposure to biohazardous material. The kit's reduced number of handling steps results in time savings and, because transfer steps of DNA containing extracts are not necessary, the cross-contamination risks are minimized.

Quality Control

- Buffered peptone water spiked with about 10⁴ cfu/ml Salmonella Enteritidis is extracted as described above.
- 5 µl of the extract is analyzed using the **food**proof *Salmonella* Detection Kit. 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.



Helpful Hint

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	8000
8000	5200
5000	2000
3000	720
1000	80

5. Supplementary Information

5.1 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.

5.2 Approvals

The **food**proof ShortPrep I Kit has been AOAC-RI and NordVal validated together with the **food**proof *Salmonella* Detection Kit (R 300 27).

5.3 Trademarks

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5.4 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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