BIOTECON Diagnostics

food*proof*® *Yersinia enterocolitica* and *Yersinia pseudotuberculosis* Detection Kit -5'Nuclease-

Kit for 64 Reactions **Order No. F 302 53**

Quick Reference Procedure

Version 1, January 2018

PCR kit for the qualitative detection of pathogenic *Yersinia enterocolitica* and *Y. pseudotuberculosis.*

A. Real-time PCR Time-Temperature Protocol

The following procedure is optimized for a real-time PCR instrument with FAM (for *Yersinia enterocolitica* detection), HEX (for *Yersinia pseudotuberculosis*) and ROX (for internal control detection) detection channels.
Program the PCR instrument before preparing the reaction mixes. The amplification is carried out according to the following temperature-time-program (for details on how to program the experimental protocol, see the operation manual of your real-time PCR cycler):

Pre-incubation	**1** cycle
Step 1:	37 °C for 4 minutes
Step 2:	95 °C for 5 minutes
Amplification	**50** cycles
Step 1:	95 °C for 5 seconds
Step 2*:	60 °C for 60 seconds

* Fluorescence detection in step 2

B. Preparation of the PCR Mix

Proceed as described below to prepare a 25 µl standard reaction.
Always wear gloves when handling the PCR vessels.

1. Thaw the solutions and, for maximal recovery of contents, briefly spin vials in a microcentrifuge before opening. Mix carefully but thoroughly by pipetting up and down.
2. In a reaction tube (0.5 – 2.0 ml, depending on the number of reactions), prepare the PCR mix by adding the following components in the order mentioned below, then mix gently but thoroughly by pipetting up and down.

The volumes indicated below are based on a single 25 µl standard reaction. Prepare the PCR mix by multiplying the amount in the "Volume" column by the number of reactions (sample and control reactions) to be cycled plus one or two additional reactions to cover pipetting losses.

Component	Volume
foodproof® *Yersinia* Master Mix, (vial 1, yellow cap)	18.0 µl
foodproof® *Yersinia* Enzyme Solution, (vial 2, red cap)	1.0 µl
foodproof® *Yersinia* Internal Control, (vial 3, white cap)	1.0 µl
Total volume	20.0 µl

3. Pipet 20 µl PCR mix into each PCR vessel.
   - For the samples of interest, add up to 5 µl sample DNA (if less than 5 µl add H_2O to 5 µl).
   - For the negative control, add 5 µl H_2O PCR-grade (vial 5, colorless cap).
   - For the positive control, add 5 µl **food**proof® *Yersinia* Control Template (vial 4, purple cap).
4. Seal the PCR vessels accurately with optical caps or sealing foil.
5. Briefly spin the PCR vessels in a suitable centrifuge.
6. Cycle the samples as described above.

***Note:** For further information please refer to: www.bc-diagnostics.com

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