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DATA SHEET

Version: 03
Revision date: 29/04/2025

1. Identification

Product name **PRImeDETECT™ Listeria monocytogenes Detection Kit**

Cat. No **FP0016** (96 reactions)

2. Description

Listeria monocytogenes are Gram-positive, rod-shaped ubiquitous bacteria that cause human listeriosis, a rare disease associated with high hospitalization and mortality rates—causing septicemia, meningitis, miscarriage, and stillbirth—that affects the elderly, pregnant women, newborns, and immunocompromised adults. The ingestion of contaminated food (such as meat, poultry, dairy, vegetables, and ready to eat products) is the main route of transmission to humans.

PRImeDETECT™ Listeria monocytogenes Detection Kit is based on amplification and detection of specific DNA fragments from ***Listeria monocytogenes*** by the real-time PCR method.
All reagents required for qPCR are provided ready to use as PCR Master Mix. The PCR Master Mix contains the appropriate amounts of buffer, dNTPs, Hot-start DNA polymerase, DNA-free water and MgCl2 to perform the number of reactions indicated in the kit. The PCR Master Mix also includes an internal amplification control (IAC) whose detection indicates the absence of PCR inhibitors. Primers and probes for the amplification of IAC as well as for the amplification of the target gene are included in the Master Mix. The probe for the detection of target gene is labelled with the FAM, whereas the probe for the detection of IAC is labelled with the HEX fluorochrome. In addition, the kit includes positive control DNA and negative control. The positive control is supplied to demonstrate that the PCR amplification is working efficiently with the supplied components. To confirm absence of contamination, a negative control reaction should be included every time the kit is used.
Include DNAready lysis buffer to extract the DNA from the sample prior to PCR detection.

3. Composition

- ✓ PCR Master Mix (1 vial)
- ✓ DNAready lysis buffer (1 bottle)
- ✓ PCR Positive Control (1 vial)
- ✓ PCR Negative Control (1 vial)

4. Features

- ✓ **Amplification limit:** 10 genomic units per reaction (100%), 2 genomic units per reaction (40%)
- ✓ **Quantification limit:** 20 genomic units per reaction (100%).
- ✓ Quantification Dynamic range: 6 logs
- ✓ **Target:** *Listeria monocytogenes*
- ✓ **Inclusivity:** Positive in 48 reference strains of L. monocytogenes (serovars 1 to 7) from different collections.
- ✓ **Exclusivity:** 100% Tested with 94 nontarget strains composed of 51 strains of *Listeria* non-*monocytogenes* and 43 strains of non-*Listeria*.
- ✓ **Detection:** probe labelled with fluorescent dyes ***Listeria monocytogenes:*** FAM-BHQ1a; **IAC:** HEXBHQ1.
- ✓ **Thermal cycler:** Agilent Mx3005P, Applied Biosystems 7300, 7500 and other cyclers.

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5. Storage specifications

The **PRImeDETECT™ Listeria monocytogenes Detection Kits** are shipped at ambient temperature. On arrival the kit should be stored at -20˚C. Avoid prolonged exposure to light. If stored correctly the kit will retain full activity for 12 months. The kit can be stored at 4˚C for 1 month.

6. Recommended Protocol

Sample preparation

For preparation of master suspensions, follow the instructions of EN ISO 6887-1 to 5 standards. Comply with Good Laboratory Practices (refer to EN ISO 7218 standard).

Regular procedure:

L. monocytogenes enrichments: Homogenize* 25 g of food sample in 225 ml of Half Fraser Broth (HF) and incubated at 37 ˚C for 26 ± 2 hours.
*Homogenize using the Stomacher or similar for 1 min. After incubation keep bags in the refrigerator (4 ºC) if step 3 cannot be followed immediately. Maximum storage time: 72 hours.

DNA extraction

Place 0.5 ml HF *(Listeria)* enrichments in a microcentrifuge tube and centrifuge at 8000 × g for 5 min. Discard the supernatant.
Use the pellet for DNA extraction using the Lysis Buffer as follows:
1. Resuspend the pellet with 100 µl of Lysis Buffer
2. Incubate at 56 ºC for 30 minutes followed by 90ºC for 10 minutes
3. Centrifuge at 8000 × g for 5 minutes.
4. Use the DNA-containing clear supernatant to load the PCR reaction.
Recommendations: Prepare 1/10 dilution and use it to load the PCR (ex.10 µl of extract + 90 µl of H2O).

PCR reaction:

Load 5 µl of the extracted DNA samples into each PCR tube or plate well containing 15 µl of the reaction mix. Load also 5 µl of the positive controls into the appropriate tubes or plate wells. Place the PCR tubes or the plate into the real-time thermal cycler. Set the fluorescence reading at the channels corresponding to the fluorochromes FAM and HEX.

Step	Time	Temperature
Initial denaturation	10 min	95˚C
40 Cycles	15 sec 1 min	95˚C 60˚C*

*Fluorescence measurements should be carried out during Step 2 at the end of each Annealing/Extension cycle at 60 ºC

7. Analysis of results

Follow instrument software instructions to generate cycle threshold (Ct) values from the acquired data.

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The quantity of DNA target in each sample can be calculated by referring to the positive control template Ct value. Please be aware the positive control is intended to verify correct assay performance and should not be used as the sole reference for quantitative analysis.

8. Further information

Product Use Limitations This product is developed, designed, and sold exclusively only for research purposes use. The product was not tested for use in diagnostics or for drug development, nor is it suitable for administration to humans or animals.

Safety Information When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, please consult the appropriate material safety data sheets (MSDS). These are available online in convenient and compact PDF format at www.canvaxbio.com where you can find, view, and print the MSDS for each CANVAX kit.