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DATA SHEET

Version: 02
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1. Identification

Product name — **PRImeDETECT™ Salmonella Plus Detection Kit**
(Salmonella spp & Listeria monocytogenes)

Cat. No — **FP0011** (96 reactions)

2. Description

Waterborne and foodborne pathogens are ubiquitous in the environment. The threat to human health posed by foodborne pathogens has attracted public attention, and the incidence of illness or death caused by major known pathogens has increased worldwide. Infections or outbreaks caused by major foodborne pathogens can be the result of consuming contaminated foods, including beef, milk products, fresh vegetables, and contaminated water. In addition, food itself is a complex system, as well as a complicated environment, which can supply the enough nutrition for the bacteria.

Canvax Biotech SL offers molecular microbiology PCRbased detection systems intended for agri-food in a userfriendly, rapid, accurate and cost-effective format.

PRImeDETECT™ Salmonella Plus Detection Kit (Salmonella *spp & Listeria monocytogenes*) is based on amplification and detection of specific DNA fragments from ***Salmonella spp*** and/or Listeria monocytogenes by the real-time PCR method (multiplex PCR).

All reagents required for qPCR are provided ready to use as Multiplex PCR Master Mix (1 vial). The Multiplex 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 Multiplex 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 (Listeria) and CY5 fluorochrome (Salmonella), 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(DNA-free water). 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

- ✓ Multiplex 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 UG per reaction (100%).
- ✓ **Quantification limit:** 20 UG per reaction (100%).
- ✓ **Quantification Dynamic range:** 6 logs
- ✓ **Simultaneously** detect and differentiate. Salmonella spp. and Listeria monocytogenes in the sam tube
- ✓ **Inclusivity:** ***Salmonella:*** Positive in 30 references strains of food origin from the Spanish Type Culture Collection (CECT). ***Listeria:*** Positive in 48 reference strains of L. monocytogenes (serovars 1 to 7) from different collections.
- ✓ **Exclusivity:** ***Salmonella:*** Tested with 29 nontarget strains. ***Listeria:*** 100% Tested with 94 nontarget strains composed of 51 strains of Listeria nomonocytogenes and 43 strains of non-Listeria.

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- ✓ **Detection:** probe labelled with fluorescent dyes–***Salmonella:*** HEX-BHQ1; ***Listeria:*** FAM-BHQ1a; **IAC:**CY5-BHQ2.
- ✓ **Thermal cycler:** Agilent Mx3005P, Applied Biosystems 7300, 7500 and other cyclers.

5. Storage specifications

The **PRImeDETECT™ Salmonella Plus Detection Kit** is 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. 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.

PROTOCOL

Sample preparation

For preparation of master suspensions, follow the instructions of EN ISO 6887-1 to 5 and EN ISO 6579 standards. Comply with Good Laboratory Practices (refer to EN ISO 7218 standard).

Regular procedure:

Salmonella food enrichments: Homogenize* 25 g of food sample in 225 ml of buffered peptone water (BPW) and incubating at 37 ˚C for 16 ± 2 hours.
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 1ml of BPW (Salmonella) and 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 ul 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 ADN-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).

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PCR reaction:

Load 5 µl of the extracted DNA samples into each PCR tube or plate well containing 15 ul 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, HEX and CY5.

PCR cycling conditions

Step	Time	Temperature
Initial denaturation	10 min	95˚C
40 Cycles	15 sec 1 min	95˚C 60˚C*
Melt analysis	Refer to instrument instructions	Refer to instrument instructions

Analysis of results

Follow instrument software instructions to generate cycle threshold (Ct) values from the acquired data. The user may also, optionally, analyze the melt profile of each reaction.
The quantity of DNA target in each sample can be calculated by referring to the positive control template Ct value.
A result will be considered as positive whenever fluorescence corresponding to Salmonella and Listeria intercepts the threshold value for detector. It is recommended to analyze each fluorescence channel separately.
A reaction will be considered negative whenever no amplification curve is produced or fluorescence does not cross the threshold and there is an amplification curve for IAC at the expected Ct.

PRECAUTIONS

- ✓ Follow ISO22174:2005 *Microbiology of food and animal feeding stuffs -- Polymerase chain reaction (PCR) for the detection of food-borne pathogens -- General requirements and definitions*
- ✓ **Good Laboratory Practice** must be observed in order to obtain reliable results with this technique. The high sensitivity of this test requires extreme care to maintain the purity of all reagents.
- ✓ Nucleic acids are very sensitive to degradation by nucleases, which are present in human skin and in surfaces that have been in contact with human skin. Wash surfaces with appropriate reagents, use powder-free examination gloves and a lab coat throughout the whole test. Wash hands thoroughly after performing the test.
- ✓ This test has been validated by using the reagents provided with **PRImeDETECT™ Salmonella plus Detection Kit.** The use of other amplification methods or any change in the protocol may render false results. DO NOT INTERCHANGE COMPONENTS from different lots.
- ✓ Do not use **PRImeDETECT™ Salmonella Detection plus Kit** after expiry or best before date. Store this product at the indicated temperature and conditions.
- ✓ The use of this product is limited to qualified personnel experienced in DNA extraction and amplification techniques.
- ✓ **Not For Medical Diagnostic Use.**