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DATA SHEET

Version:03
Revision date: 03/06/2025

1. Identification

Product name **PRImeDETECT™ Salmonella Detection Kit**

Cat. No **FP0021** (96 rxn)

2. Description

Salmonella and Campylobacter are the most frequently isolated foodborne pathogens, and are predominantly found in poultry, eggs and dairy products. Salmonella infection remains a major public health concern worldwide, contributing to the economic burden of both industrialized and underdeveloped countries through the costs associated with surveillance, prevention and treatment of disease. Gastroenteritis is the most common manifestation of Salmonella infection worldwide, followed by bacteremia and enteric fever.

PRImeDETECT™ Salmonella Detection Kit (*Salmonella spp*) is based on amplification and detection of specific DNA fragments from ***Salmonella spp*** 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 Cy5, 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 (2 vials, 48 rxn each)
- ✓ DNAready lysis buffer (2 bottles, 48 rxn each)
- ✓ 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:** Salmonella spp
- ✓ **Inclusivity:** 100% Inclusivity. > 700 Salmonella strains tested, including S.enterica and S. bongori
- ✓ **Exclusivity:** 100%. Tested with 42 strains of the rest of Enterobacteriaceae and Eubacteria
- ✓ **Detection:** probe labelled with fluorescent dyes. **Salmonella spp:** Cy5-BHQ1; **IAC:** HEX- BHQ1.
- ✓ **Thermal cycler:** Agilent Mx3005P, Applied Biosystems 7300, 7500 and other cyclers.

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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 and EN ISO 6579 standards. Comply with Good Laboratory Practices (refer to EN ISO 7218 standard).

Regular procedure:

Prepare a 1/10 initial suspension of the food. Typically, 25 g or 25 ml of food in 225 ml of Buffered Peptone Water (BPW). Homogenize using the Stomacher or similar for 1 min.

Bacterial enrichment

Incubate BPW-containing bag under static conditions at 37 °C, 18 ± 2 hours. After incubation keep bags in the refrigerator (4 °C) if step 3 cannot be followed immediately. Maximum storage time: 72 hours.

DNA extraction

Place 1ml of BPW 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 °C for 30 minutes followed by 95 °C for 10 minutes
3. Centrifuge at 8000 × g for 5 minutes.
4. Use the DNA-containing clear supernatant to load the PCR reaction.

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 Cy5, HEX (IAC).

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PCR cycling conditions

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 °C

Analysis of results

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

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 intercepts the threshold value for detector.
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 ISO 22174:2024 / UNE-EN ISO 22174:2025 Microbiology of the food chain – Polymerase chain reaction (PCR) for the detection and quantification of microorganisms and viruses – 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 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 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**