Data sheet

PRImeDETECT™ Salmonella Detection Kit

(Salmonella spp)

Cat. No: FP0020 (48 reactions)
Cat. No: FP0021 (96 reactions)

Introduction

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 (1 vial). The PCR Master Mix contains the appropriate amounts of buffer, dNTPs, Hot-start DNA polymerase, DNA-free water and $MgCl_2$ 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 JOE 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.

Each kit contains:

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

Shipping and Storage

The **PRImeDETECT™ Salmonella 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.

Technical features

- ✓ **Amplification limit:** 10 UG per reaction (100%), 2 UG per reaction (40%)
- ✓ **Quantification limit:** 20 UG 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:** FAM-BHQ1a; **IAC**: JOE-TAMRA.
- ✓ **Thermal cycler:** Agilent Mx3005P, Applied Biosystems 7300, 7500 and other cyclers.

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 ºC, 18 ± 2 hours.
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 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 95 ºC for 10 minutes
3. Centrifuge at 8000 × g for 5 minutes.
4. Use the ADN-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 FAM, JOE (IAC).

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	

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

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 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 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 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**.