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PCR-Salmonella Detection Kit

(Catalog# K1447-96; 96 Rxns; Storage at -20°C)

I. Introduction:

PCR-Salmonella Detection Kit is an ideal tool for a fast and reliable amplification and detection of specific DNA fragments from Salmonella spp. by the real-time PCR method. The Kit includes all the reagents required in a comfortable ready-to-use Multiplex PCR MasterMix. The optimized MasterMix contains a Buffer, dNTPs, Hot-start DNA Polymerase, DNA-free water, $MgCl_2$ and 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 MasterMix. The probe for the detection of target gene is labeled with FAM, whereas the probe for the detection of IAC is labeled with another fluorochrome. In addition, the kit includes both Positive Control 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. The DNAready Lysis Buffer is included to extract the DNA from the sample prior to PCR Detection.

II. Applications:

- For fast, reliable amplification and detection of specific DNA fragments from Salmonella spp. by the real-time PCR method.

III. Sample Type:

- Poultry, eggs and dairy products

IV. Kit Contents:

Components	K1447-96	Part Number
PCR Master Mix	1 Vial	K1447-96-1
DNAready Lysis Buffer	1 Bottle	K1447-96-2
PCR Positive Control	1 Vial	K1447-96-3
PCR Negative Control	1 Vial	K1447-96-4

V. User Supplied Reagents and Equipment:

- Stomacher or similar
- Centrifuge; Water bath
- PCR tube
- Buffered Peptone Water (BPW)

VI. Storage Conditions and Reagent Preparation:

All the reagents are shipped in dry ice and stored at -20°C upon receipt. Avoid prolonged exposure to light. If stored correctly the kit will retain full activity for 12 months.

VII. Assay Protocol:

Sample Preparation & PCR Protocol:

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 37ºC, 18 ± 2 hours.
After incubation keep bags in the refrigerator (4 ºC) if DNA extraction cannot be followed immediately. Maximum storage time: 72 hours.
DNA extraction: Place 1ml of BPW enrichments in a microcentrifuge tube and centrifuge at 8000g 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 56ºC for 30 minutes followed by 95ºC for 10 minutes
3. Centrifuge at 8000g for 5 minutes.
4. Use the clear supernatant to load the PCR reaction.

PCR reaction: Load 5 µl of the 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, IAC.
PCR cycling conditions:

Step	Time	Temperature
Initial Denaturation	10 min	95°C
40 Cycles	15 sec	95°C
	1 min	60°C
Melt Analysis	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 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.

VIII. Related Products: DNA extraction kits (Cat# K1411-K1417); K309; K967

FOR RESEARCH USE ONLY! Not to be used on humans.