Instructions for use
InviScreen® *Salmonella* spp. Detection Kit

INVITEK diagnostics

InviScreen®

Language: EN

REF 6015007200

Σ 100 tests

ALS Life Sciences Portugal, S.A.
Zona Industrial de Tondela, ZIM II,
Lote 6, 3460-070 Tondela, Portugal

IMPORTANT NOTES

Thank you for purchasing the InviScreen® *Salmonella* spp. Detection Kit from Invitek Diagnostics.

The product serves the purpose of manual isolation of DNA from bacteria in pre-enriched food samples and detection of *Salmonella* spp. by real-time PCR.

WARNING! Improper handling and use for other than the intended purpose can cause danger and damage. Therefore, we ask you to read through these instructions for use and follow them carefully. Always keep them handy. To avoid personal injury, also observe the safety instructions.

All versions of the instructions for use can be found on our website for download or can be requested from us: **invitek.com**

CONTACTS

Technical support:
techsupport@invitek.com

PORTUGAL

Zona Industrial de Tondela, ZIM II, Lote 6, 3460-070 Tondela, Portugal
+351 232 817 817

GERMANY

Robert-Rössle-Str. 10, 13125 Berlin, Germany

INVITEK diagnostics

TABLE OF CONTENT

1. INTRODUCTION

One of the most common causes of foodborne illness is *Salmonella*, a genus of bacteria responsible for an infection called salmonellosis. Salmonellosis is a serious public health concern throughout the world. Release of contaminated foods can result in processing delays, product recalls, plant shutdowns, and legal action. It can cost food companies millions in revenue as well as their reputation, stock value, and future profitability.

A key to preventing *Salmonella* contamination is timely and accurate food pathogen testing. To be effective, a *Salmonella* test must detect low levels of the bacteria in a wide range of food types quickly, easily, and with high specificity. Traditionally, *Salmonella* testing has been performed using culture-based or immunoassay methods, which are time-consuming and labor-intensive. With culture-based methods, for example, the time-to-results can take up to 5 days, and interpretation of the results is highly subjective. Immunoassay-based methods can take up to 2 days and may lack specificity due to antibody binding interferences. In this context, the use of molecular biology technology by resorting to DNA testing have been highlighted. Real-Time PCR technology allows DNA amplification with robustness, sensitivity, and specificity.

2. INTENDED USE

The InviScreen® *Salmonella* spp. Detection Kit is specifically designed for the qualitative detection of *Salmonella* spp. in food samples. This kit is intended for use by trained laboratory professionals, providing a sensitive and reliable method for the detection of *Salmonella* spp. in a broad range of food products, including meat products, poultry products, dairy products, egg products and fresh produce and fruits. Laboratory personnel should follow the instructions provided in this manual carefully, including the sample preparation and pre-enrichment, DNA extraction, Real-Time PCR amplification and result interpretation guidelines. This *Salmonella* spp. Real-Time PCR detection kit is a high-quality molecular biology solution to provide the general laboratory and research personnel an easy and quick ready-to-use kit for the detection of *Salmonella* spp. in different food categories in one and a half hours after a one-step pre-enrichment, taking **less than 10 hours in total** for the majority of matrices.

3. PRODUCT DESCRIPTION

The InviScreen® *Salmonella* spp. Detection Kit method uses the real-time PCR technique to detect the presence of *Salmonella* spp. DNA in food products intended for human consumption. The assay utilizes a fast extraction method and is based on the amplification of specific DNA using hydrolysis probes. The kit allows simultaneous amplification of the target DNA and an internal amplification control (IAC) to exclude false negative results due to PCR inhibition. This duplex detection system helps maximize amplification accuracy and simplify reaction setup. This real-time PCR method uses the commonly available FAM and VIC/HEX fluorescence channels. Enrichment culture media is not provided with the kit.

4. GENERAL WORKFLOW

This method is aimed to allow the qualitative detection of *Salmonella* spp. in food products samples previously enriched by culture. Pre-enrichment, DNA extraction and Real-Time PCR are the three main steps involved:

5. KIT CONTENTS AND STORAGE

One InviScreen® *Salmonella* spp. Detection Kit contains sufficient reagents for **100 tests**, according to the table below.

REF	COMPONENT	FUCTION	CAP COLOR	QUANTITY	STORAGE (UPON ARRIVAL)
D41.01	PRIMER/PROBE MIX	TARGETED DETECTION	**BROWN**	1 TUBE, 1000 µL	-20 ± 5°C
D41.02	QPCR MASTER MIX	AMPLIFICATION	**GREEN**	1 TUBE, 1000 µL	-20 ± 5°C
D41.03	NEGATIVE CONTROL	NEGATIVE CONTROL	**COLOURLESS**	1 TUBE, 100 µL	-20 ± 5°C
D41.04	POSITIVE CONTROL	POSITIVE CONTROL	**RED**	1 TUBE, 100 µL	-20 ± 5°C
D41.05	FAST LYSIS REAGENT	DNA EXTRACTION	**COLOURLESS**	1 BOTTLE, 20 ML	ROOM TEMPERATURE

Fast lysis reagent should be stored at room temperature. The other reagents should be stored sealed at -20 ± 5°C. All reagents may be used until the expiration date shown on the package label. Expiry date refers to the product under rightful handling and storage conditions. It is not recommended the use of the kit after the expiry date stated on the box. Avoid unnecessary repeated freeze/thawing cycles (>5). Protect reagents from light exposure to prevent degradation.

6. EQUIPMENT AND MATERIALS REQUIRED (NOT PROVIDED)

- Stomacher bags (preferably with incorporated filter)
- Scale/dilumate
- Laboratory paddle blender for homogenizing test samples
- Buffered Peptone Water (BPW, ISO formulation)
- Incubator operating at 37 ± 1 ºC
- Microbiology Pipettes and tips (1000 µL)
- 1.5 mL microcentrifuge tubes (preferably with lock cap)
- Microcentrifuge capable of speed >10.000 x g
- Vortex mixer
- Dry bath capable of maintaining 99 °C and high-speed agitation
- Micropipettes and micropipette filter tips (1000 µL, 200 µL or 100 µL, 10 µL)
- White qPCR tubes and optical caps
- PCR cabinet to minimize the risk of contamination with foreign DNA
- Real-Time PCR instrument (Bio-Rad CFX96 Deep Well PCR and Applied Biosystems QuantStudio 5 RT PCR System (0.1 mL, 96-well block))

7. SAFETY PRECAUTIONS AND RECOMMENDATIONS

Anyone using this product should have received instructions in general safety practices for laboratories and the safety information provided in this document.

- The kit is designed for use by trained professionals in laboratory settings.
- The user assumes all responsibility for compliance with local regulations and guidelines for the use of this product.
- Samples and enrichment cultures must be handled as potentially infectious material and discarded according to local regulations.
- All potentially infectious material should be autoclaved (e.g. 121 ºC for 20 min) before disposal.
- Every procedure should be performed in strict compliance with Good Laboratory Practices (example EN ISO 7218) especially concerning PCR.
- It is advised to follow the general requirements described in EN ISO 22174:2024 - Microbiology of the food chain - Polymerase Chain Reactions (PCR) for the detection and quantification of microorganisms - General requirements and definitions.

8. PROTOCOL

The procedure includes three main steps: pre-enrichment, DNA extraction and Real-Time PCR amplification.

PRE-ENRICHMENT

1. Pre-warm the BPW to the sample incubation temperature (37 ± 1 °C) before use.
2. Add 225 mL of pre-warmed BPW to 25 g/25 mL of sample **(1:10 dilution ratio)**.

   Note 1: The use of enrichment bag with incorporated filter is highly recommended.
3. Homogenize for **30 seconds in a stomacher**.
4. Incubate without shaking at **37 ± 1 °C for 8 to 16 hours** or **16 to 24 hours** according to the tables below.

   Note 2: It is possible to store the enriched samples at 2-8 °C for 72 hours maximum, following the incubation at 37 °C, for the Raw and Ready-to-Eat (RTE) Meat Products, RTE and Ready-to-Reheat (RTRH) Poultry Products and Eggs and Egg Products. For Heat Processed and Raw Milk and Dairy Products, Raw Poultry Products and Fresh Produce and Fruits, following enrichment, proceed to the Fast Lysis DNA Extraction.
5. Proceed to the **Fast Lysis DNA Extraction**.

AOAC PTM #102401		
MATRICES	**SAMPLE PREPARATION**	**ENRICHMENT**
Raw milk (25 mL)	Homogenize 25 mL of sample in 225 mL of pre-warmed BPW (1:10 dilution)	Incubate 8-16 hours at 37 ± 1 °C
Raw ground beef (25 g)	Homogenize 25 g of sample in 225 mL of pre-warmed BPW (1:10 dilution)	Incubate 8-16 hours at 37 ± 1 °C
Raw ground turkey (25 g)	Homogenize 25 g of sample in 225 mL of pre-warmed BPW (1:10 dilution)	Incubate 8-16 hours at 37 ± 1 °C
Deli turkey (25 g)	Homogenize 25 g of sample in 225 mL of pre-warmed BPW (1:10 dilution)	Incubate 8-16 hours at 37 ± 1 °C
Cut cantaloupe (25 g)	Homogenize 25 g of sample in 225 mL of pre-warmed BPW (1:10 dilution)	Incubate 16-24 hours at 37 ± 1 °C
Pasteurized liquid egg (25 mL)	Homogenize 25 mL of sample in 225 mL of pre-warmed BPW (1:10 dilution)	Incubate 16-24 hours at 37 ± 1 °C

MICROVAL 2023LR127 (pending MicroVal approval)		
MATRICES	**SAMPLE PREPARATION**	**ENRICHMENT**
Heat Processed and Raw Milk and Dairy Products	Homogenize 25 g/25 mL of sample in 225 mL of pre-warmed BPW (1:10 dilution)	Incubate 16-24 hours at 37 ± 1 °C
Raw and Ready- to-eat (RTE) Meat Products	Homogenize 25 g of sample in 225 mL of pre-warmed BPW (1:10 dilution)	Incubate 8-16 hours at 37 ± 1 °C
Raw Poultry Products	Homogenize 25 g of sample in 225 mL of pre-warmed BPW (1:10 dilution)	Incubate 8-16 hours at 37 ± 1 °C
RTE and RTRH Poultry Products	Homogenize 25 g of sample in 225 mL of pre-warmed BPW (1:10 dilution)	Incubate 8-16 hours at 37 ± 1 °C
Fresh Produce and Fruits	Homogenize 25 g of sample in 225 mL of pre-warmed BPW (1:10 dilution)	Incubate 16-24 hours at 37 ± 1 °C
Eggs and Egg Products	Homogenize 25 g/25 mL of sample in 225 mL of pre-warmed BPW (1:10 dilution)	Incubate 16-24 hours at 37 ± 1 °C

FAST LYSIS DNA EXTRACTION

6. Label **1.5 mL** microcentrifuge tubes.
7. Take **1 mL of the enriched sample** from the corresponding bag to each tube.

   Note 3: Open bags and tubes carefully to avoid possible cross contamination.

8. Centrifuge **1 mL aliquots** tubes at max. speed (>10.000 x g) for 2 min and carefully discard the supernatant. Use a 1000 µL micropipette, if necessary (make sure that all liquid is properly removed. If needed use a 100 µL pipette to fully remove all liquid).

   Note 4: Enriched samples with a lot of food residues should be pre-cleared by centrifuging up to 2 mL of sample at 900 x g for 2 minutes. Then, use 1 mL of supernatant and centrifuge as described above in a fresh tube.

   Note 5: For enriched samples showing a significant fat layer after the first centrifugation step, carefully remove fat together with supernatant.

   Note 6: If necessary, pellets can be frozen at -20 °C in case DNA extraction has to be repeated.

9. Release the pellet from the bottom of the tube by vortex or scraping the tube across the top of a tube rack.

10. Add **200 µL Fast Lysis reagent** to the tube.

    Note 7: Shake the buffer well before use.

11. Vortex vigorously for **10 seconds**.

12. Incubate for **10 min at 99 °C** on a dry bath with constant shaking (e.g. 1.400 rpm).

13. Cool down at **room temperature for ≈2 min**.

14. Centrifuge for **2 min at max. speed (>10.000 x g)**.

15. Carefully transfer **100 µL of the supernatant**, without disturbing the pellet, to a clean tube. If carryover of sample pellet occurs and/or cell debris are still visible in the supernatant, repeat steps 14 and 15, to obtain a clearer supernatant.

16. The supernatant (DNA extract) can be stored at 4 °C up to 1 week or at -20 °C for longer periods.

17. Proceed to **Real-Time PCR detection of *Salmonella* spp**.

REAL-TIME PCR DETECTION OF *SALMONELLA* SPP.

PREPARATION OF THE PCR SETUP

All DNAs extracted from each sample should be analysed following the analytical conditions described below. For each Real-Time PCR run, at least one positive control reaction and one negative control reaction must be included. Allow all reagents of the *Salmonella* spp. Real-Time PCR detection kit to thaw at 2-8 °C, vortex and spin briefly to avoid drops on the vial cap.

18. For each sample prepare a reaction mix according to the table below:

REAGENT	VOLUME
PRIMER/PROBE MIX	10 µL
QPCR MASTER MIX	10 µL
TOTAL VOLUME	20 µL

19. Prepare the number of reactions needed according with the table in Annex A. An extra volume of at least one reaction should be prepared to account for pipetting deviations.

20. Homogenize the reaction mixture and pipette **20 µL** into individual wells according to the predicted qPCR plate or tubes set up.

21. Add **5 µL of DNA extract** to each well.

22. For the positive control reaction and negative control reaction, replace the sample in these wells with **5 µL of Positive Control** (Red cap) and **5 µL of Negative Control** (Colourless cap), respectively.

    Note 8: It is recommended to prepare the reaction mixture carefully in a controlled environment, preferably in a nucleic acid-free zone.

    Note 9: The addition of the positive control and sample DNA extract should preferably be carried out in a separate room.

AMPLIFICATION PROTOCOL

The amplification conditions for both validated instruments are as follows:

STEP	TEMPERATURE	TIME	CYCLE
ENZYMATIC ACTIVATION	95 ºC	2 MIN	1
DENATURATION	95 ºC	1 SEC	40
HYBRIDIZATION/EXTENSION PLATE READING	62 ºC	20 SEC	

Fluorescence data must be obtained during this step through FAM (target DNA) and HEX/VIC (internal amplification control, IAC) channels. The assay was validated on Bio-Rad CFX96 Deep Well PCR and Applied Biosystems QuantStudio 5 RT PCR System (0.1 mL, 96-well block).

RESULTS INTERPRETATION

Analysis parameters should be set in accordance with the analysis software recommended by the Real-Time PCR instrument manufacturer. Automatic Baseline Threshold settings per instrument manufacturer's instructions should be used. The analysis outcome should be interpreted according to the scenarios referred bellow.

The result can only be considered valid under the following control conditions:

CONTROLS	*Salmonella* DNA (FAM)	IAC (HEX/VIC)
POSITIVE CONTROL	CT ≤ 26	+ (POSITIVE)
NEGATIVE CONTROL	- (NEGATIVE)	+ (POSITIVE)

If **no amplification** is observed for the positive control, the test results are invalid and must be repeated.

If **amplification** is observed for the negative control, it indicates that the reagents have become contaminated while setting up the run, invalidating test results. In each case, repeat the Real-Time PCR reaction.

The reaction includes an **IAC (HEX/VIC)** to monitor the reaction and to exclude the occurrence of PCR inhibition. A poor signal obtained for IAC indicates the presence of PCR inhibitors in the reaction which compromises an effective and accurate detection test.

Salmonella DNA amplification is monitored in FAM channel and a result is considered positive when **CT ≤ 35** for the tested DNA.

CONTROLS	*Salmonella* DNA (FAM)	IAC (HEX/VIC)
POSITIVE SAMPLE	CT ≤ 35	+ (POSITIVE) / - (NEGATIVE)
NEGATIVE SAMPLE	- (NEGATIVE)	+ (POSITIVE)
INCONCLUSIVE	- (NEGATIVE)	- (NEGATIVE)
INCONCLUSIVE	? (INCOMPLETE AMPLIFICATION CURVE)	+ (POSITIVE) / - (NEGATIVE)

An **inconclusive** test may be due to the presence of excessive DNA and/or **PCR inhibitors**. It is recommended to **perform a 1:2 and 1:10 dilution of the DNA extract** in DNAse/RNAse free water and repeat the Real Time PCR reaction. See Annex B for troubleshooting.

9. CONFIRMATION OF POSITIVE RESULTS

A positive InviScreen® *Salmonella* spp. Detection Kit is presumed to be positive and confirmation according to an appropriate reference method (example: ISO 6579) is recommended. Confirmation of presumptive positive results may be conducted according to ISO 6579 from enrichments stored at 2-8°C for a maximum of 72 hours for Raw and Ready-to-Eat (RTE) Meat Products, RTE and Ready-to-Reheat (RTRH) Poultry Products and Eggs and Egg Products. For Heat Processed and Raw Milk and Dairy Products, Raw Poultry Products and Fresh Produce and Fruits, confirmation shall begin following enrichment. Plate primary enrichments using the ISO 6579 secondary enrichment procedure and incubate as appropriate. After incubation, inspect the plates for typical colonies and confirm their identity using appropriate techniques.

10. PERFORMANCE VALIDATION

PERFORMANCE TESTED AOAC RESEARCH INSTITUTE LICENSE NUMBER 102401

The InviScreen® *Salmonella* spp. Detection Kit is certified by the AOAC Research Institute under PTM #102401 for the detection of *Salmonella* spp. in raw milk (25 mL), raw ground beef 80% lean (25 g), raw ground turkey (25 g), deli turkey (25 g), cut cantaloupe (25 g), and liquid pasteurized egg (25 mL). A positive result with the InviScreen® *Salmonella* spp. Detection Kit is presumed to be positive and confirmation according to an appropriate reference method is recommended. All sample types were validated on Bio-Rad CFX96 Deep Well PCR and Applied Biosystems QuantStudio 5 RT PCR System (0.1 mL, 96-well block).

ANNEX A | REAL-TIME PCR MIX CALCULATION GUIDE

To find the correct volumes to use when preparing the PCR mix, add the total number of samples and controls to be analysed and find the corresponding volumes of master mix and primer/probe mix in the table.

NUMBER OF REACTIONS	qPCR MASTER MIX (µl)	PRIMER/PROBE MIX (µl)	FINAL VOLUME (µl)
1	10	10	20
2	20	20	40
3	30	30	60
4	40	40	80
5	50	50	100
6	60	60	120
7	70	70	140
8	80	80	160
9	90	90	180
10	100	100	200
11	110	110	220
12	120	120	240
13	130	130	260
14	140	140	280
15	150	150	300
16	160	160	320
17	170	170	340
18	180	180	360
19	190	190	380
20	200	200	400
21	210	210	420
22	220	220	440
23	230	230	460
24	240	240	480
25	250	250	500
26	260	260	520
27	270	270	540
28	280	280	560
29	290	290	580
30	300	300	600
31	310	310	620
32	320	320	640
33	330	330	660
34	340	340	680
35	350	350	700
36	360	360	720
37	370	370	740
38	380	380	760
39	390	390	780
40	400	400	800
41	410	410	820
42	420	420	840
43	430	430	860
44	440	440	880
45	450	450	900
46	460	460	920
47	470	470	940
48	480	480	960

ANNEX B | TROUBLESHOOTING

PROBLEM	POSSIBLE CAUSE	RECOMMENDATION
Low amount of nucleic acids	Bacterial pellet lost	Avoid disturbing the pellet when removing the supernatant.
	Incorrect storage of starting material	Ensure that starting material is appropriately stored. Avoid repeated thaw-freeze cycles of the sample material.
Degraded nucleic acids	Incorrect storage of starting material	Ensure the sample is taken and stored correctly.
	Old material	Ensure the sample is taken and stored correctly.
Nucleic acids do not perform well in downstream applications (e.g. PCR)	Inhibition	Dilute eluate in PCR grade water (e.g., 1:2 or 1:10).
	Carryover of sample pellet	When transferring the eluate to a fresh tube, strictly avoid transferring any pelleted material from the bottom of the tube.
A poor signal obtained for IAC	Inhibition	Dilute eluate 1:2 and/or 1:10 in PCR grade water.
Incomplete amplification curves	Low amount of DNA template	Repeat the Real-Time PCR for result confirmation.

invitek.com

INVITEK
diagnostics

PORTUGAL
Zona Industrial de Tondela, ZIM II, Lote 2 e 6
3460-070 Tondela
Portugal

Phone: +351 232 817 817

GERMANY
Robert-Rössle-Str. 10
13125 Berlin
Germany

info@invitek.com
www.invitek.com

DE 552.03
23-10-2024