

Instruction Manual

DUPLIC α REAL TIME SALMONELLA

Code EBR004032 Format 32 tests

For in Vitro Use Only

Store the kit components at +2 - 8°C

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I- INTRODUCTION

Salmonellosis is an infection with a bacteria called Salmonella. Most persons infected with Salmonella develop diarrhea, fever, and abdominal cramps 12 to 72 hours after infection. The illness usually lasts 4 to 7 days, and most persons recover without treatment. However, in some persons the diarrhea may be so severe that the patient needs to be hospitalized. In these patients, the Salmonella infection may spread from the intestines to the blood stream, and then to other body sites and can cause death unless the person is treated promptly with antibiotics. The elderly, infants, and those with impaired immune systems are more likely to have a severe illness.

The Salmonella germ is actually a group of bacteria that can cause diarrheal illness in humans. They are microscopic living creatures that pass from the feces of people or animals, to other people or other animals. There are many different kinds of Salmonella bacteria. Salmonella serotype Typhimurium and Salmonella serotype Enteritidis are the most common in the United States. Salmonella has been known to cause illness for over 100 years. They were discovered by a American scientist named Salmon, for whom they are named.

Many different kinds of illnesses can cause diarrhea, fever, or abdominal cramps. Determining that Salmonella is the cause of the illness depends on laboratory tests that identify Salmonella in the stools of an infected person. These tests are sometimes not performed unless the laboratory is instructed specifically to look for the organism. Once Salmonella has been identified, further testing can determine its specific type, and which antibiotics could be used to treat it. (Centre for disease Control and Prevention; <http://www.cdc.gov>)

DUPLIC α ^{RealTime} for Salmonella spp was designed to identify a specific fragment of the PFKB-THRS intergenic region.

II- DUPLIC α ^{RealTime} TECHNOLOGY

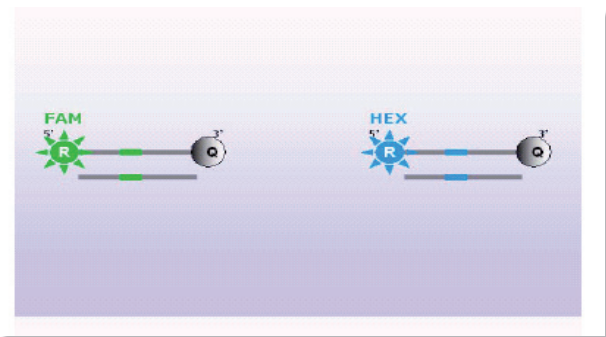
DUPLIC α ^{RealTime} Salmonella spp test is based on gene amplification and a fluorogenic probe is used for the detection of Salmonella spp DNA. The reagents for the amplification are ready to use and provided with 3 reactions mix:

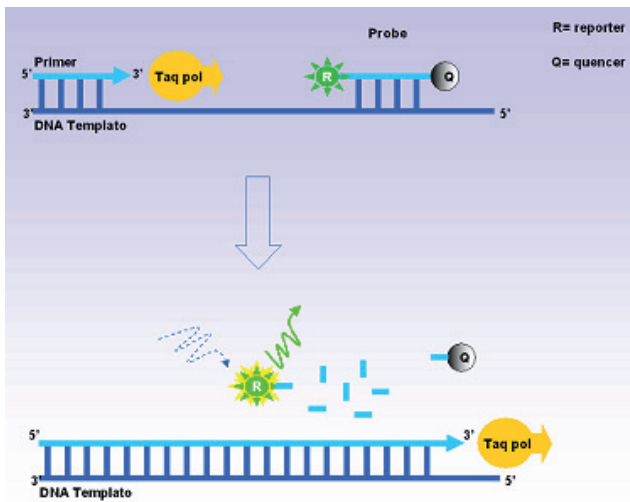
- AMPLIFICATION MIX: with Hot Start Taq DNA polymerase, (BlueTaq DNA polymerase, Euroclone), nucleotides, MgCl₂ and buffer.
- OLIGO MIX with primers and fluorogenic probes.
- INTERNAL CONTROL: for evaluate the amplification reaction.

In the PCR procedure trace amounts of DNA can be quickly and repeatedly copied to produce a quantity sufficient to investigate using conventional laboratory methods. There are three basic steps involved in the PCR process: denaturation, annealing, extension. Denaturation to promote single-strandedness of the template DNA is achieved by heating to approximately 95°C. The temperature is then lowered significantly to promote base-pairing (annealing) of the primer to the template. The temperature is then shifted to the optimum temperature for the DNA polymerase enzyme to synthesize sequences complementary to the template using the annealed primer as a starting point for the extension of the newly synthesized strand.

In a PCR-Real time system fluorescent probes can be used for an on line detection of DNA amplification

Fig. 1





Test is based on gene amplification and a fluorogenic probe is used for the detection of *Salmonella* spp DNA. This assay allows for the direct detection of specific PCR products within minutes of completion of the PCR by monitoring the increase in fluorescence of a dye-labelled oligonucleotide probe.

Each probe consists of an oligonucleotide with a 5'-reporter dye and a 3'-quencher dye. FAM (6-carboxy-fluorescein) is covalently linked to the 5'-end of the probe for detection of DNA target. HEX (hexachlorofluorescein) is covalently linked to the 5'-end of the probe for detection of internal control (IC). Each of these two reporters are quenched by BHQ (black hole quencher) linked through a linker arm on the 3'-end of each probes. Quencher can quench the reporter fluorescent only when the two dyes are close to each other. This happens when the probe is intact. Once amplification occurs, the probe is destroyed by the 5'-3'

exonuclease activity of the Taq DNA polymerase and the fluorescence will be detected by the optical system (Fig.2). The fluorescence detected by instrument increased together with the DNA amplification.

III -DUPLIC α .RealTime ADVANTAGES

Easy to use: The Oligo mix and Amplification mix tubes of Kit include all the reagents for PCR: the Master-Mix is added directly to the purified sample to perform PCR reaction.

Real Time PCR general advantages:

- Real Time PCR allows a highly sensitive quantification of the gene of interest
- This technology combines DNA amplification with detection of the products in a single tube.
- Less time consuming than gel based analysis
- Minimized risk of cross contamination
- High throughput and automatic process possibility
- It removes the significant contamination risk caused by opening tubes for post-PCR-manipulation.

IV - KIT CONTENTS

Reagents	Code colore
Amplification mix	Blu Cap
Oligo Mix*	Green Cap
Control 1 (Positive Control)	Red Cap
Control 2 (Internal Control)	Yellow Cap
Blank (B)	White Cap

* keep the tubes away from light

The kit is performing 32 reactions, in each session the extracted DNA must be co-amplified with internal control to identify inhibitions in the reactions of amplification.

The kit is for 4 sessions with 6 samples, 1 Positive Control (C1) and one blank of reaction (B).

V - STORAGE AND STABILITY

Twelve months at 2-8°C.

VI- MATERIALS REQUIRED BUT NOT PROVIDED

Equipments and materials required for DNA extraction

- 1.5 ml tubes
- Micropipette 1000 μ l
- Micropipette 10-100 μ l
- Plugged tips
- Rack for 1.5 ml tubes
- Centrifuge (12 000 rpm)
- Incubator
- Vortex

Materials required for amplification:

- Micropipette 1-10 µl
- Micropipette 10-100 µl
- Plugged tips
- Rack for 0.2 ml tubes
- Optical microplate for real Time PCR
- Tubes of 0.2 ml with optical caps
- Thermalcycler for Real Time PCR

PRECAUTIONS

- The kit can be used after a training for instruction of use
- Equipments and material must not be moved from a working area to another room.
- Don't use the reagents after date of expiration.
- Mix the reagents of kit before used.
- Periodically verify the calibration of micropipette and the operative of instruments.
- Frequently change the gloves.
- Periodically wash a working area with clorox 5%
- Used gloves without powder, don't make a fingerprints on optical caps. Don't write on caps because it possible overlapping with fluorescences detection.

VIII - PROTOCOL**a) Sample preparation**

The Real Time Salmonella spp Detection System enables the user to reveal the presence of Salmonella in foodstuff or raw materials after a pre-enrichment step with Buffered Peptone water for 18 ± 2 hours at 37 ± 1 °C (ISO6579)

b) Genomic DNA Extraction

Any commercial DNA extraction kit could be used. The extraction matrix is the bacterial culture

c) Real time PCR cycler programming

Refer to the specific handbook of the equipment used but be sure to set the following thermal profile

Time	Temperature	N° Cycles
10 min	95°C	1
20 sec	95°C	50
60 sec	60°C	

d) PCR mix preparation

For each experiment make a master mix for: control, 1 reaction blank, n+1 samples. The reagents of the mix have to mixed under this ratio:

REAGENT	VOLUME (µl)
Amplification mix	10
Oligo mix	10
Control 2 (Internal Control)	1
DNA	4

After a mix preparation, aliquot 21µl of Master Mix in the tubes or in the PCR microplates than add in each tube 4µl from the Extracted DNA, put tubes into the equipment and start the program of amplification already set. At the end of the program remove the tubes from the thermocycler

e) Interpretation of results

Interpretation of results are made by a software specific of the RT thermocycler used for amplification. The fluorescence in each channel indicate the hybridation of the probe

Channel 1 for FAM= DNA Target probe
Channel 2 for HEX: Internal control probe.

Fluorophore FAM (Listeria)	Fluorophore HEX (I.C.)	RESULT
Ct > 0	Not relevant (Ct ≥ 0)	POSITIVE
Ct = 0	Ct > 0	NEGATIVE
Ct = 0	Ct = 0	INHIBITION

IX- SENSITIVITY

The sensitivity of the test is 1-5 Genomic DNA Copies/test (the sample volume is 4ul)

X- TROUBLESHOOTING

No fluorescent signal can be monitored

1. Wrong channel has been chosen.
2. Pipetting errors or omitted reagents
3. Inhibitory effect of the samples: genomic DNA with a insufficient purification and or insufficient extraction
4. Check for correct conservation of the kit.
5. Control the performances of the thermalcycler.

Fluorescence intensity is too low

1. Deterioration of dyes and or primers in the device due to unsuitable storage condition
2. Very low starting amount of genomic DNA and/or low purity.

Fluorescence intensity varies

1. The prepared PCR master mix is not well mixed
2. Air bubble is trapped in the PCR tubes

REFERENCES

1. Molecular And Cellular Probes, 15, 275–280
2. Applied and Environmental Microbiology, 61(10), 3745–3747

Euroclone Related Products

Code	Product description	N°
EBR003032	Duplicα ^{Real time} Lysteria monocytogenes detection Kit	32 test