

Instruction Manual

***DUPLIC $\alpha$  REAL TIME  
LISTERIA MONOCYTOGENES  
DETECTION KIT***

***Code EBR003032 Format 32 tests***

***For in Vitro Use Only***

Store the kit components at +2 - 8°C

***Euro@lone<sup>®</sup>***

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## I- INTRODUCTION

*Listeria monocytogenes* has been recognized as a human pathogen for 60 years, but food was not thought to be a vehicle of transmission until recently. Pregnant women, newborns, and adults with weakened immune systems are primarily at risk. About one-third of *Listeria monocytogenes* cases occur during pregnancy.

*Listeria monocytogenes* is found in soil and water. Vegetables can become contaminated from the soil or from manure used as fertilizer. Animals can carry the bacteria without appearing ill and can contaminate foods of animal origin such as meats and dairy products. The bacteria can be found in a variety of raw foods, such as uncooked meats and vegetables, as well as in processed foods that become contaminated after processing, such as soft cheeses and cold cuts at the deli counter. Unpasteurized (raw) milk or foods made from unpasteurized milk may contain the bacteria.

Symptoms vary and depend on the individual's susceptibility, but may include fever, fatigue, nausea, vomiting and diarrhea.

This is a Gram-positive bacterium, motile by means of flagella. Some studies suggest that 1-10% of humans may be intestinal carriers of *L. monocytogenes*. It has been found in at least 37 mammalian species, both domestic and feral, as well as at least 17 species of birds and possibly some species of fish and shellfish. It can be isolated from soil, silage, and other environmental sources. *L. monocytogenes* is quite hardy and resists the deleterious effects of freezing, drying, and heat remarkably well for a bacterium that does not form spores. Most *L. monocytogenes* are pathogenic to some degree.

*L. monocytogenes* has been associated with such foods as raw milk, supposedly pasteurized fluid milk, cheeses (particularly soft-ripened varieties), ice cream, raw vegetables, fermented raw-meat sausages, raw and cooked poultry, raw meats (all types), and raw and smoked fish. Its ability to grow at temperatures as low as 3°C permits multiplication in refrigerated foods.

DUPLIC $\alpha$ <sup>RealTime</sup> *Listeria monocytogenes* was designed to identify a specific fragment of the HLY A gene.

## II -DUPLIC $\alpha$ <sup>RealTime</sup> TECHNOLOGY

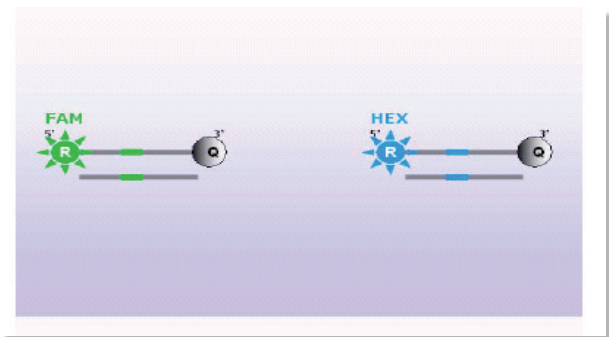
DUPLIC $\alpha$ <sup>RealTime</sup> *Listeria monocytogenes* Test is based on gene amplification and a fluorogenic probe is used for the detection of *Listeria monocytogenes* DNA. The reagents for the amplification are ready to use and provided with 3 reactions mix:

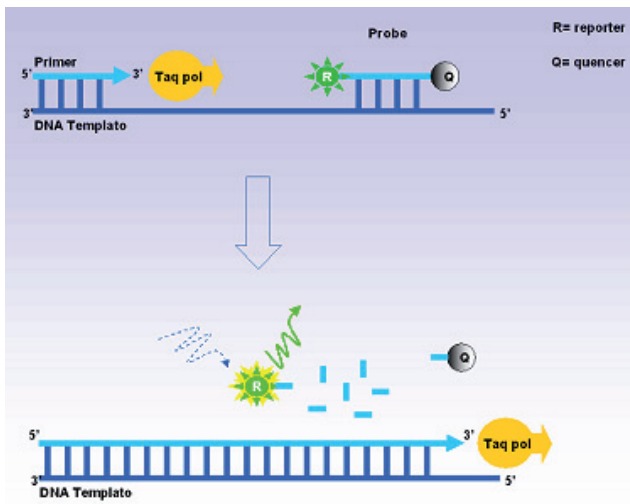
- AMPLIFICATION MIX: with Hot Start Taq DNA polymerase, (BlueTaq DNA polymerase, Euroclone), nucleotides, MgCl<sub>2</sub> and buffer.
- OLIGO MIX with primers and fluorogenic probes.
- INTERNAL CONTROL: to evaluate the amplification reaction.

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 *Listeria monocytogenes* 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 $\alpha$ 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

### 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  $\mu$ l
- Micropipette 10-100  $\mu$ 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 *Listeria monocytogenes* Detection System enables the user to reveal the presence of *Listeria* in foodstuff or raw materials after a pre-enrichment step with Fraser ½ medium for 24 hours at 30°C (ISO11290-1).

**b) Genomic DNA Extraction**

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

**c) Real time PCR cyclyer 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 ( <i>Listeria</i> )	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°
EBR004032	Duplicα <sup>Real time</sup> Salmonella detection Kit	32 test