



Via Sant'Anna 131-135  
61030 Cartoceto PU (IT)  
Telephone + 39 (0)721830605  
Fax +39 (0)721837154  
e-mail: info@diatheva.com  
www.diatheva.com

## *L. monocytogenes* and *Salmonella* spp. FLUO kit

**REF** MBK0053 - 50 Tests

### INTRODUCTION AND PRODUCT DESCRIPTION

Foodborne diseases are a serious global health problem. Microbial food safety is an increasing public health concern worldwide. Each year, as many as 600 million, or almost one in 10 people in the world, fall ill after consuming some sort of contaminated food. Several outbreaks of food-borne illnesses following consumption of several food caused by *Salmonella* spp. and *L. monocytogenes* have been reported in recent years, indicating the importance of this problem in safeguarding public health (CDC, 2015). Conventional bacteriological methods are often long and tedious.

The *L. monocytogenes* and *Salmonella* spp. FLUO kit is intended for the simultaneous molecular detection of *Salmonella* spp. and *L. monocytogenes*, by multiplex Real-time PCR. The kit uses DNA primers and fluorescent probes specific for the target organisms. If pathogens are present, DNAs are amplified and the increased fluorescence signals are recorded in Real-Time. The internal amplification control, presents in the amplification mix, assesses the efficiency of each amplification reaction by checking the presence of inhibitory factors and ensuring reliability of negative results. Results are obtained within few hours following an enrichment step and subsequent DNA extraction.

The *L. monocytogenes* and *Salmonella* spp. FLUO kit provides a ready-to-use Master Mix dedicated for the use with the instruments with three and four-plex capability, and positive control.

### KIT CONTENTS

Component	MBK0053
1X Master Mix	2 x 0.5 ml
Positive Control (10 <sup>5</sup> cells for each target species/5 µl)	1 x 0.1 ml
PCR Grade Water	1 x 1 ml
ROX	2 x 5 µl

### STORAGE

Store the kit at -20°C upon arrival, protect from light. If properly stored, see the expiration date for the stability of the kit.

### ADDITIONAL EQUIPMENT

- Gloves
- Pipette and pipette tips with aerosol preventive filter
- 1.5 ml microcentrifuge tubes
- 0.2 ml PCR tubes
- Vortexer
- Microcentrifuge

### PRECAUTIONS AND RECOMMENDATIONS

- The test must be performed by specialised, trained and authorised staff.
- Do not use reagents after the expiry date printed on the label.
- Use gloves as well as sterile pipet tips with filters. Change gloves often, especially if you suspect a possible contamination of them.
- It is suggested to provide separate and dedicated spaces, material and equipment for pre and post-PCR amplification stages.
- Clean working space periodically with at least 5% sodium hypochlorite or another decontaminant agent.

## 1. PROCEDURE

### 1.1 DNA ISOLATION

#### *Food samples*

For sample preparation please refer to manual of Bacterial DNA Isolation Single Step (Diatheva cod. MBK0063-MBK0076).

### Colony

- Dispense 100 µl of DNase free water in a 1.5 ml tube and dissolve the colony.
- Boil the sample for 10 minutes.
- Centrifuge at 14 000 rpm for 10 minutes.
- Collect the supernatant in a 1.5 ml tube taking care to do not disrupt the pellet.
- Mix and use 2 µl of the sample in the Real-Time PCR reaction.

## 1.2 PCR MIX PREPARATION

All detection experiments should include an NTC (No Template Control), containing all the components of the reaction except for the template. This enables detection of potential contamination. Moreover include at least one positive control.

Total volume PCR reaction is 25 µl.

- Thaw the components (1X Master Mix, PCR Grade Water, ROX and Positive Control), protect from light. Vortex 15" and centrifuge briefly.

For instruments that require a passive reference dye add ROX to 1X Master Mix immediately before the use, according to the instructions below:

-For Low ROX instruments (i.e.: ABI 7500, QuantStudio 3-5) → add 15 µl of the PCR Grade Water to the vial containing the 5 µl ROX, vortex for 30" and centrifuge briefly. Proceed by completing the 1X Master Mix with the addition of 0.56 µl of diluted ROX.

-For High ROX instruments (i.e: ABI 7900) → the ROX provided in the kit is ready-to-use (no dilution is required). Proceed by completing 1X Master Mix with the addition of 1.56 µl ROX.

*Note: the MBK0053 kit provides 2 separate vials of ROX, one for each 1X Master Mix vial. It is recommended to dilute the ROX and to complete the 1X Master Mix just before the use. The diluted ROX cannot be stored after the preparation.*

- Vortex for 15" the prepared 1X Master Mix and centrifuge briefly.
- Aliquot 20 µl of 1X Master Mix in 0.2 ml tubes or alternatively in the plate prepared for the experiment;
- Add 5 µl of PCR Grade Water into NTC;
- In a separate area, add 5µl of DNA samples to be tested into the corresponding PCR tubes or wells containing amplification mix;
- Add 5 µl of Positive control into the corresponding PCR tubes or wells containing amplification mix;
- Seal hermetically the PCR tubes or plate and load into the real-time PCR instrument, following the manufacturer's instructions.

*Note: for PCR instrument with 96 wells block check that the reagents are at the bottom of each well, if not, briefly centrifuge at 800 x g for 1 minute.*

Optimal instrument and fluorescence analysis settings are a prerequisite for accurate results. For details, please refer to the manual provided with your Real-Time PCR instrument. Otherwise it could be used also with other instruments provided with acquisition channels listed below.

## 1.3 PROGRAM SETUP

Program the Real-Time PCR instrument according to the operator's manual and the table below:

STEP	TEMPERATURE	TIME	CYCLES
Initial denaturation	95°C	3 min	1X
Denaturation	95°C	20 sec	45X*
Annealing/Extension	63°C	1 min	

Acquire on the GREEN (FAM), YELLOW (VIC), RED (Quasar 670), channels during Annealing/Extension; For Rotor-Gene Q instrument that allows the gain optimization on the acquiring channels, set the gain optimization on NTC sample (tube position 1).

\*Select 50X for peltier block instruments.

Select ROX as passive reference dye for instruments that require it (es. Applied Biosystems).

**Please note:** Verify that fluorophores available for the acquisition channel listed in the table above are calibrated. Select non fluorescent quencher.

Target species	Acquisition channel
<i>Salmonella</i> spp.	Green, FAM (Ex 495-Em 520 nm)
<i>L. monocytogenes</i>	Red, Cy5, Quasar 670 (Ex 647-Em 670nm)
Internal Amplification Control	Yellow, VIC (Ex 538-Em 554 nm)

**1.4 ANALYSIS SETUP**

The analysis of the results should be done with the program included in the recommendations provided by the manufacturer of the instrument. For a correct definition of the threshold it is necessary to select a value distinction from the background after the linear phase growth. Analyse each sample in the three acquisition channels.

**1.5 INTERPRETATION OF RESULTS**

**Controls:** Before interpreting sample results, it is necessary to verify the positive and negative controls. For the experiment to be valid, the controls must have the following results:

	<i>Salmonella</i> spp. (Green)	<i>L. monocytogenes</i> (Red)	Internal Amplification Control (Yellow)
<b>Negative Control</b>	No amplification	No amplification	20≤Ct≤30
<b>Positive Control</b>	15≤Ct≤25	15≤Ct≤25	Not significant*

\*The amplification in this channel may also not be present

**Samples:** check that the curves are typical amplification curves. If the Ct value in the Green, or Red channel is ≤10, verify in the raw data that the curve is a regular amplification curve. If correct the sample could be considered positive for *Salmonella* spp. or *L. monocytogenes*, respectively.

Target*	Internal Amplification Control	Interpretation
No amplification	20≤Ct≤32	Negative
No amplification	No amplification	Inhibition**
Ct≥10	Not significant	Positive

\* *Salmonella* spp. *L. monocytogenes*

\*\*In case of inhibition is necessary dilute sample and repeat a further PCR

**1.6 CONFIRMATION OF POSITIVE RESULTS**

All positive PCR results need to be confirmed according to the reference method ISO 11290 (for *L. monocytogenes*) and ISO 6579 (for *Salmonella* spp.)

**TROUBLESHOOTING**

No signal, poor Rn value (PCR) or signal detected late in PCR	Pipetting error or missing reagent	Check the storage conditions of the reagents, Repeat the assay.
	Problems with starting template DNA	Check the concentration, storage conditions, and quality of the template and control DNA.  Efficient removal of PCR inhibitors is essential for optimal results. Purify nucleic acids from your sample using an appropriate purification method.  Insufficient or degraded template DNA, increase the amount of template DNA if possible.

**REFERENCES**

**Centers for Disease Control (CDC). 2015.** Surveillance of foodborne diseases outbreaks in the United States, 2013: annual report. Atlanta, Georgia: US Department of Health and Human Services, CDC