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Salmonella spp. FLUO kit

REF MBK0054 – 50 Reactions

REF MBK0057 – 100 Reactions (2x MBK0054)

INTRODUCTION AND PRODUCT DESCRIPTION

Salmonellosis is the second most commonly reported gastrointestinal infection and an important cause of foodborne outbreaks in the EU/EEA (ECDC, 2019). The fast and accurate identification of *Salmonella* spp. from food samples insures not only a better quality of products, but also the possibility to adopt timely precautionary measures to limit the spread of infection in case of an outbreak.
Conventional assays in common use can take up to days or more.
The salmonella spp FLUO kit is intended for the rapid detection of Salmonella DNA. The kit uses DNA primers and fluorescent probe specific for the target organism. If pathogen is present, DNA is amplified and the increased fluorescence signals are recordered in Real-Time. The internal control, present in the amplification mix, assesses the efficiency of amplification reaction by checking the presence of inhibitory factors and ensuring reliability of negative results. A multiplex assay with two dyes is used: probes for the target DNA and the internal control, each labeled with different fluorophores are in the same tube. Results are obtained within a few hours following an enrichment step and subsequent DNA extraction.

KIT CONTENTS

Component	MBK0054	MBK0057
1X Master Mix	2 x 0.5 ml	4 x 0.5 ml
DNA polymerase (5U/µl)	1 x 15 µl	2 x 15 µl
Positive Control (10^5 cells for the target specie/5 µl)	1 x 0.2 ml	2 x 0.2 ml
PCR grade water	1 x 1 ml	2 x 1 ml
ROX	2 x 5 µl	4 x 5 µl

ADDITIONAL EQUIPMENT

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

STORAGE

The product should be stored immediately upon arrival at -20 °C.
If properly stored, see the expiration date for the stability of the kit.

PRECAUTIONS

The user should always pay attention to:

- use pipette tips with aerosol-preventive filters, deionized DNA-free water and gloves;
- store positive material (specimens, controls and amplicons) separately from all other reagents and if possible, add it to the reaction mix in a separated space;
- do not use the same precision pipettes for reaction mix and DNA;
- thaw all components and samples at room temperature before starting an assay, when thawed, mix the components and centrifuge briefly;
- do not use reagents after their expiration date
- verify the accuracy and precision of pipettes, as well the correct functioning of instruments;
- clean works space periodically with at least 10% bleach or other decontaminant agent.

1. PROCEDURE

Food, environmental and primary production samples

1.1 DNA ISOLATION

For sample preparation please refer to manual of Fast DNA Extraction kit (Diatheva, Cod. MBK0061) or Bacterial DNA Isolation Single Step (Diatheva cod. MBK0063-MBK0076).

Colony

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

1.2 PCR SET-UP

All detection experiments should include an NTC (No-Template Control), containing all the components of the reaction except for the template. Moreover include at least one positive control. Total volume per reaction is 25 µl.

- Thaw the components protect from light. Vortex Master Mix for 10" and centrifuge briefly.
- Upon first use of Master Mix, for instruments that require a passive reference dye (ex. Applied Biosystems), it is necessary to add the ROX.

- Low ROX: 7500, 7500 fast, QuantStudio 3-5
- High ROX: ABI Prism® 7000- 7300, 7700-7900HT StepOne and StepOne plus

Dilute the ROX (5 µl) by the addition of DNase free water according to the type of Real-Time PCR thermal cycler:

Instrument	**Low ROX**	**High ROX**
Volume of DNAse free water	195 µl	15 µl

Note: it is recommended to dilute the ROX just before use, and directly into the vial of ROX provided. The kit provides separate vials of ROX for each vials of Master Mix provided in the kit.

- Vortex for 20" and centrifuge briefly.
- Proceed by completing Master Mix (directly in the vial in which is provided) with the addition of ROX or DNAse free water according to the following scheme.

	For 1 vial	**For 1 vial**	**For 1 vial**
Real-Time PCR Instrument	**Low ROX**	**High ROX**	**NO ROX**
Multiplex PCR Master Mix	500 µl	500 µl	500 µl
ROX diluted	6.2 µl	6.2 µl	-

In one sterile 1.5 ml tube prepare the amplification reaction mix needed for each sample to be tested plus one NTC and one Positive Control following the pipette scheme below:

	1 reaction*
Master Mix	19.8 µl
DNA Polymerase (5U/µl)	0.2 µl
Total volume	20 µl

*For the analysis of more than one sample, simply multiply the volumes of Master Mix and DNA Polymerase for the number of samples to be tested considering the NTC and Positive Control.

- Vortex for 15" the vial containing the prepared PCR master mix and centrifuge briefly;
- Aliquot 20µl of PCR 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;

1.3 THERMAL PROFILE

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. The kit has been optimized to be used with Rotor-Gene Q (Qiagen), Rotor-Gene 6000 (Corbett) and ABI 7500 (Applied Biosystems) instrument. Otherwise it could be used also with other instruments provided with acquisition channels listed below.
Program the Real-Time PCR instrument as indicated below according to the operator's manual:

STEP	**TEMPERATURE**	**TIME**	**CYCLES**
Initial denaturation	95°C	1 min	1X
Denaturation	95°C	20 sec	40X*
Annealing/Extension	63°C	1 min	
Acquire on the GREEN (FAM) and YELLOW (VIC) channels during Annealing/Extension; if possible, set the gain optimization on NTC sample before 1st acquisition.			

*****45 cycles for instrument based on Peltier block technology (ex. 7500 Applied Biosystems)

Please note: if your instrument requires ROX as passive reference, select ROX as passive reference dye. Verify that dyes available for the acquisition channels listed above are calibrated. Select non fluorescent quencher.

Target	**Acquisition channel**
Salmonella spp.	Green, FAM (Ex 495-Em 520nm)
Internal Amplification Control	Yellow, VIC (Ex 538-Em 554nm)

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 four 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:

	***Salmonella* spp. (Green)**	**Internal Amplification Control (Yellow)**
Negative Control	No amplification	20≤Ct≤36
Positive Control	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 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.

Target	**IAC**	**Interpretation**
No amplification	20≤Ct≤36	Sample negative
No amplification	No amplification	Inhibition
Ct≥10	Positive/negative	Sample positive

In case of partial or complete 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 6579 included Amendment 1, Annex D/2007 for primary production samples.

FLOW DIAGRAM OF CONFIRMATION STEP of PCR positive results

Procedure for foods and environmental sample (excluding primary productions samples)		Procedure for primary production sample
Streak 10 µl onto chromogenic formulation agar plate ↓ Incubate for 24 h ± 3 h at 37°C ± 1°C ↓ Observe characteristic colonies of *Salmonella* spp. ↓ Confirm by using latex test onto an isolated colony	Subculture in RVS broth 0.1 ml/10 ml ↓ Incubate for 24 h ± 3 h at 41.5°C ± 1°C ↓ Streak onto XLD and a chromogenic formulation agar plate ↓ Incubate for 24 h ± 3 h at 37°C ± 1°C ↓ Observe characteristic colonies of *Salmonella* spp. ↓ Confirm by using latex test onto an isolated colony	0.1 ml in 2-3 spots onto MSRV agar ↓ Incubate for (2x) 24 h ± 3h at 41.5°C ± 1°C ↓ Streak onto XLD and a chromogenic formulation agar plate ↓ Incubate for 24 h ± 3 h at 37°C ± 1°C ↓ Observe characteristic colonies of *Salmonella* spp. ↓ Confirm by using latex test onto an isolated colony
RVS- Rappaport-Vassiliadis medium with soya XLD- Xylose lysine deoxycholate agar MSRV-Modified semi-solid Rappaport-Vassiliadis agar		

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

ECDC. 2019. Salmonellosis epidemiological report for 2016