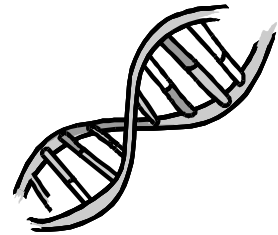


MutaREAL[®]

Salmonella sp.

real time PCR Kit



Screening test for the *real time* detection of *Salmonella sp.* in food samples using the capillary system of the LightCycler[®] from Roche.

KV2901124 (24 determinations)
KV2901196 (96 determinations)

According to § 35 LMBG (Lebensmittel/Bedarfsgegenständegesetz)

Immundiagnostik AG, Stubenwald-Allee 8a, 64625 Bensheim, Germany
info@immundiagnostik.com
Tel.: +49 (0)6251/ 70190-0
Fax: +49 (0)6251/ 849430
www.immundiagnostik.com

1. INTENDED USE

The MutaREAL® *Salmonella sp. real time* PCR Kit is a qualitative screening assay for the detection of *Salmonella* species in food samples using the capillary system of the LightCycler® (Roche).

2. INTRODUCTION

Salmonella has been one of the most frequently occurring foodborne pathogen affecting the microbial safety of foods, including egg and egg-products as well as meat and poultry (approx. 65% of all cases). So far, more than 2000 serovars have been identified, all potentially pathogenic for human. For this reason the European Determinations prescribe that in 25 g of food no *Salmonellae* should be detected.

Compared to the conventional bacteriologic methods, which are very time-intensive and expensive, the MutaREAL *Salmonella sp. real time* PCR is a fast, qualitative test, that is based on amplification and specific detection of *Salmonella sp.* DNA isolated from many different food types. The test gives a result within one hour after pre-enrichment.

3. PRINCIPLE OF THE TEST

The *real time* PCR Kit MutaREAL® *Salmonella sp.* contains specific primers, hybridization probes and additional material for the detection of *Salmonella species* in food samples.

The assay uses a thermostable DNA polymerase to amplify a specific gene fragment by means of PCR (polymerase chain reaction). Furthermore in the same step proof of specificity is achieved in *real time* by hybridization of the amplicon with specific hybridization probes after which as consequence, fluorescence is emitted and measured by the LightCycler®'s optical unit. All different food samples, such meat, egg products, dough samples etc. can be used. The detection of amplified *Salmonella sp.* DNA is performed in fluorimeter channel F2.

Furthermore by the use of an internal control that is included in each reaction and that is co-amplified and detected, a possible inhibition of the reaction can be determined. The detection of amplified internal control is performed in channel F3.

4. KIT CONTENT

Each kit contains enough reagents to perform 24 respectively 96 tests. Each kit also contains a package insert.

Ref.	Type of reagent	Presentation 24	Presentation 96	Cap color
A1a	Enzyme-Mix	1 vial, 4 µl	3 vials, 4 µl	blue
A1b	Enzyme buffer (with dNTP)	1 vial 60 µl	3 vials, 60 µl	blue
A2	Primer-/ Probe-Mix	1 vial, 300 µl	1 vial, 1.1 ml	yellow
A3	MgCl ₂ (25mM stock solution)	1 vial, 250 µl	1 vial, 1 ml	white
A4	Positive control	1 vial, 20 µl	1 vial, 50 µl	red
A5	Negative control	1 vial, 200 µl	1 vial, 200 µl	green

5. TEST PERFORMANCE

Required materials - provided:

- PCR reagents
- Package insert

Required materials - not provided:

- LightCycler® instrument (Roche)
- LightCycler® capillaries (Roche)
- LightCycler® capillary centrifuge (Roche)
- LightCycler® Color Compensation kit (Roche)
- LightCycler® Cooling Block (Roche)
- DNA extraktion kit
- Pipets (0.5 – 1000 µl)
- sterile filter tips for micro pipets
- sterile microtubes
- table centrifuge

6. STORAGE AND HANDLING

- All reagents (A1 to A5) should be stored at -20°C.
- All reagents can be used until the expiration date printed on the labels.
- Do not freeze and thaw the reagents A1 (a, b), A2, A3, A4 several times.
- Use LightCycler® Cooling Block (Roche) or cool all reagents during the working steps.
- Primer-/ Probe-Mix (A2) should be stored in the dark.

7. WARNINGS AND PRECAUTIONS

- According to §35 LMBG (Lebensmittel-/Bedarfsgegenständegesetz)
- This assay needs to be carried out by skilled personnel
- Food samples should be regarded as potentially infectious materials
- This assay needs to be run according to GLP (Good Laboratory Practice)
- Do not use the kit after its expiration date
- The LightCycler® PCR process is owned by Hoffmann-La Roche Ltd.

AMPLIFICATION

The PCR technology is utmost sensitive. Thus, amplification of a single molecule generates millions of identical copies. These copies may evade through aerosols and sit on surfaces. In order to avoid contamination of samples with DNA which previously was amplified, it is important to physically strictly divide sample and reagent preparation units from sample amplification units. Set up two separate working areas:

- 1) Isolation of the DNA
- 2) Amplification/ detection of amplification products

Pipets, vials and other working materials should not circulate among working units!

- Use always sterile pipette tips with filters
- Wear separate coats and gloves in each area
- Routinely decontaminate your pipettes and the laboratory benches with decontaminant
- Avoid aerosols

8. PROCEDURE

The complete procedure is separated in four steps:

- A) Pre-enrichment and DNA extraction
- B) Preparation of the ready-to-use enzyme mix
- C) Amplification and combined detection of DNA templates using FRET-hybridization probes (LightCycler® PCR)
- D) Interpretation of the results using the LightCycler® software

A) PREENRICHMENT AND DNA-EXTRACTION

1. Preenrichment of 25 g food sample (meat should be homogenized) in 225 ml of buffered peptone water is done by incubation of sample in preenrichment solution (prewarmed peptone water) at 37°C for 18 +/- 2 h (in stomacher without mixing).
2. For DNA-extraction 1 ml of preenriched food sample is centrifuged (12000 rpm for 2 min) and obtained pellet is used as template. Extraction is done using a commercially available DNA-isolation kit (suited for food samples) according to the manufacturer's instructions.
3. If the LightCycler® PCR is not performed immediately, store the extracted DNA at -20°C.

B) PREPARATION OF THE *READY TO USE* ENZYME MIX

1. Centrifuge shortly 1 vial enzyme mix (A1a) and 1 vial enzyme buffer (A1b) at 13000 rpm.
2. Transfer enzyme buffer (A1b) quantitatively (60 µl) into enzyme mix (A1a).
3. Mix carefully by pipetting (**do NOT vortex!**). This is the *ready to use* Enzyme Mix.

C) *REAL TIME* Salmonella sp. PCR-PROTOCOL

Please carefully read the manufacturer's instructions before starting the procedure!

The Master Mix volume for the respective number of samples and controls should be pipetted as follows:

1. The Enzyme Mix volume per reaction and sample (N) should be multiplied with number of samples to be performed, including controls A4 and A5. For reasons of un-precise pipetting, add an extra (virtual) sample. Proceed in the same manner with all additional reagents!

Reaction Volume	Master Mix Volume
2 µl Enzyme Mix (<i>ready to use</i>) (A1a + b)	2 µl x (N+1)
10.6 µl Primer-/ Probe Mix (A2)	10.6 µl x (N+1)
2.4 µl MgCl ₂ (A3)	2.4 µl x (N+1)

Mix gently (**do NOT vortex!**) the following reagents in a sterile tube: Enzyme Mix (*ready to use*; A1a + A1b), Primer-/ Probe Mix (A2) and MgCl₂ (A3). This mixture is the Master Mix - spin down briefly in a table centrifuge.

2. Pipet **15 µL** of Master Mix using micropipets with sterile filter tips in each of the LightCycler capillaries. Add **5 µL** of the DNA sample or positive and negative controls (A4 and A5) to each of these capillaries (It is recommended to pipet the negative control first to avoid contamination). Immediately lock capillaries to avoid contamination.

Spin down briefly (in a LightCycler® capillary centrifuge).

Perform the following LightCycler® PCR protocol:

95°C for **10 min**

95°C for **10 sec** **40 cycles**
50°C for **10 sec** ramping time: 20°C/sec – aqu. mode here: SINGLE
72°C for **20 sec**

40°C for **30 sec**

D) PCR-ANALYSIS AND INTERPRETATION OF RESULTS

1. Perform the LightCycler® PCR.
2. Switch on the colour compensation filter (required because of the simultaneous use of two differently labelled hybridization probes) (by activating the field *Choose CCC File*)
3. The result for *Salmonella species* quantification is shown in channel F2/F1, the internal control in channel F3/F1.

4. Following results can arise:

- A signal is detected in channel F2/F1.
The result is positive: The sample contains *Salmonella species* DNA.

In this case, the detection of a signal in channel F3/F1 is inessential, as high concentrations of *Salmonella species* DNA can lead to a reduced or absent fluorescence signal of the internal control in channel F3/F1 (competition).

- In channel F2/F1 no signal is detected, only in channel F3/F1 (signal of the internal control).
The sample does not contain any *Salmonella species* DNA.

The detected signal of the internal control excludes the possibility of an inhibition.

- Neither in channel F2/F1 nor in channel F3/F1 is a signal detected.
A diagnostic statement can not be made.
Inhibition of the PCR reaction.

