# VetMAX™ S. enterica spp. Kit

TaqMan® real-time PCR for detecting *Salmonella enterica* spp.

**Catalog Numbers** SALMSPP, SALMSPP50

**Doc. Part No.** 100020433 **Pub. No.** MAN0008868 **Rev.** B.0

| Technology | Species | Nucleic acid isolated from matrices | Test type |
|---|---|---|---|
| Real-time PCR (DNA)<br>– Duplex<br>– Exogenous IPC | Poultry | Cloacal swabs and footpads<br>Bedding, bottom cage compartments, and soil<br>Organs and embryos | Individual |
| | Bovines | Organs (placenta biopsy, fetus)<br>Swabs (cervical, placental)<br>Vaginal mucus<br>Feces<br>Milk | Individual |

⚠ **WARNING!** Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from **thermofisher.com/support.**

⚠ **WARNING! POTENTIAL BIOHAZARD.** Read the biological hazard safety information at this product's page at **thermofisher.com.** Wear appropriate protective eyewear, clothing, and gloves.

## Information about the product

### Description of the product

The **Applied Biosystems™ VetMAX™ S. enterica spp. Kit** is a molecular diagnostic tool for detecting all *Salmonella enterica* spp. serovars through real-time PCR at animal rearing sites as well as the surrounding areas. These serovars are isolated from different samples taken during rearing site inspection or from specimens of animal origin—ruminants, poultry, porcines. (A list of the serovars is available upon request from Technical Support.)

Each DNA sample obtained after extraction is analyzed in a single well: the same well is used for specific detection of the bacterial DNA of *Salmonella enterica* spp. and for the detection of an IPC (Internal Positive Control). A positive IPC signifies both successful extraction and the absence of inhibitor in the samples.

The kit can be used on bacterial DNA extracted from poultry samples (**cloacal swabs, footpads, bedding**, bottom cage compartments, **soil, organs**, and **embryos**) or from bovine samples (**placenta biopsies, fetus, cervical or placental swabs**, **vaginal mucus, milk**, and **feces**).

Complete protocols for bacterial DNA extraction from these matrices are available upon request from Technical Support.

### Kit contents and storage

The **VetMAX™ S. enterica spp. Kit** contains components that can be used for detecting both *Salmonella enterica* spp. and an internal control IPC. Upon receipt, the whole kit should be stored between **–30°C and –10°C**. After initial use of a component, store it according to the following recommendations:

| Component | Description | Volume | | Storage | |
|---|---|---|---|---|---|
| | | SALMSPP (100 reactions) | SALMSPP50 (50 reactions) | Upon receipt | After initial use |
| 1 - Sequences Salmo (Green tube) | Sequence pool (primers and probes). Contains:<br>• The detection system for the *Salmonella enterica* spp. target, including a TaqMan® probe labeled **FAM™ - NFQ** (Non-Fluorescent Quencher).<br>• The detection system for IPC, including a TaqMan® probe labeled **VIC™ – TAMRA™**. | 2 × 100 µL | 100 µL | –30°C to –10°C | –30°C to –10°C |
| 2 - Master Mix Salmo (White tube) | Mix for TaqMan® real-time PCR. Contains the buffer and the real-time PCR enzyme. | 2 × 625 µL | 625 µL | –30°C to –10°C | 2°C to 8°C |
| 4a - EPC Salmo (Brown tube) | **External Positive Control:**<br>Positive control for *Salmonella enterica* spp. It consists of **already extracted** nucleic acid to be amplified during real-time PCR. | 2 × 90 µL | 90 µL | –30°C to –10°C | –30°C to –10°C |
| 5 - IPC Salmo (Yellow tube) | **Internal Positive Control:**<br>Exogenous internal control **to be added to each sample and each control** in the lysis step of the extraction. | 500 µL | 500 µL | –30°C to –10°C | –30°C to –10°C |

**NOTE:** For small extraction series, it is recommended that the IPC Salmo be aliquoted upon first use to avoid more than 3 cycles of freezing/thawing (a minimum volume of 50 µL).

### Extraction and amplification controls

The **VetMAX™ S. enterica spp. Kit** contains two controls, enabling validation of the extraction and amplification of the bacterial DNA.

#### 4a - EPC Salmo: positive control for *Salmonella enterica* spp.

**Already extracted** positive control to be amplified during real-time PCR.

A positive result within the specified $C_t$ range validates the amplification of the *Salmonella enterica* spp. target by real-time PCR.

#### 5 - IPC Salmo: internal extraction control

Positive control **to be added to each sample in the lysis step** of the nucleic acid extraction.

A positive result within the specified $C_t$ range in a sample validates the extraction of this sample, whether positive or negative for the target pathogen, thus eliminating false negatives and verifying the effect of inhibitors.

**We recommend including two negative controls to confirm correct analysis:**

#### NCS: negative extraction control

This control consists of reagents used in the extraction, without addition of the sample (the sample volume can be replaced by the buffer used in the sample preparation or by DNase/RNase-free water). The control undergoes the same treatment as the samples, namely nucleic acid extraction and real-time PCR.

A negative result for *Salmonella enterica* spp. confirms proper lysis progression and the absence of contamination during both extraction and real-time PCR.

#### NC: negative amplification control

This is the amplification mix deposited on the plate during the preparation of the real-time PCR, with 5 µL of DNase/RNase-free water added to adjust the reaction to 25 µL.

A negative result for *Salmonella enterica* spp. and IPC confirms the absence of contamination during real-time PCR reaction preparation.

### Materials required but not provided

Unless otherwise indicated, all materials are available through **thermofisher.com**.

- Adjustable micropipettes (range of 1 µL to 1000 µL) with DNase/RNase-free filtered tips
- DNase/RNase-free water
- 1X TE buffer
- 1X PBS buffer
- A real-time PCR thermal cycler capable of detecting the following fluorophores:
  - FAM™ (emission maximum: λ515 nm)
  - VIC™ (emission maximum: λ554 nm)
- Optical-quality consumables compatible with the thermal cycler used: PCR 96-well plates, PCR strips (8 or 12 wells), microtubes or capillaries; suitable plate covers or caps for capping

## Analysis procedure

The real-time PCR reaction volume is 25 µL:

- **Mix Salmo**: 20 µL per reaction. To be reconstituted extemporaneously before real-time PCR.
- **Extracted DNA:** 5 µL per reaction.

### Extraction of bacterial DNA

DNA must be isolated from the samples for real-time PCR analysis.

Add **5 µL** of **5 - IPC Salmo** to each sample to be extracted and to the NCS in the lysis step of the nucleic acid extraction.

**NOTE:** For information about extraction methods that are compatible with and validated for the VetMAX™ S. enterica spp. Kit, please contact Technical Support.

### Reconstitution of the reaction mix

Reconstitute the **Mix Salmo** just before use, in a room dedicated to preparation of the mix:

1. On first use, thaw **2 - Master Mix Salmo** at **2°C to 8°C, on ice** or on a refrigerated rack. Store and maintain at **2°C to 8°C** for each subsequent use.
2. Thaw **1 - Sequences Salmo** at room temperature. Return it to between **−30°C and −10°C** after use.
3. Reconstitute the reaction mix **Mix Salmo** at **2°C to 8°C, on ice** or on a refrigerated rack according to the following calculation tables:

| Component | For 1 reaction | For N reactions[1] |
|---|---|---|
| **1 - Sequences Salmo** | 2 µL | N × 2 µL |
| **2 - Master Mix Salmo** | 12.5 µL | N × 12.5 µL |
| DNase/RNase-free water | 5.5 µL | N × 5.5 µL |
| Total volume | 20 µL | N × 20 µL |

[1] It is recommended to allow for an additional reaction with respect to the total number of reactions to be carried out during the analysis (samples and controls). Never mix reagents from different lots of kits (see Certificate of Analysis).

4. After reconstitution, start the real-time PCR immediately. Keep the **Mix Salmo** at **2°C to 8°C** on ice or on a refrigerated rack until use.

### Preparation of the real-time PCR

1. Create an analysis plan for the distribution of mixes and samples. Keep the positive control (EPC) away from the other samples if possible.
2. Mix **Mix Salmo** by gentle agitation, then centrifuge briefly.
3. Add **20 µL of Mix Salmo** to each PCR plate well, PCR strip or capillary used.
4. Add the DNA from samples and controls to the mix solution, according to the following preset analysis plan:

| Type of analysis | Component | Sample volume |
|---|---|---|
| Sample for analysis | DNA extracted from the sample | 5 µL |
| Positive amplification control | **4a - EPC Salmo** | 5 µL |
| Negative extraction control (NCS) | Extracted NCS | 5 µL |
| Negative amplification control (NC) | DNase/RNase-free water | 5 µL |

5. Cover the PCR plate, PCR strips or capillaries with an adhesive plate cover or suitable caps.

### Amplification by real-time PCR

1. Create the following detectors on the thermal cycler:

| | Reporter | Quencher |
|---|---|---|
| SALM | FAM™ | NFQ (Non-Fluorescent Quencher) |
| IPC SALM | VIC™ | TAMRA™[1] |
| Passive reference: ROX™[1] | | |

[1] The TAMRA™ and ROX™ fluorophores are obligatory for real-time PCR analysis if the thermal cycler is capable of detecting them. For other thermal cyclers, absence of the ability to detect these fluorophores does not compromise the analysis by real-time PCR.

2. Assign the **Salmo** detector and the **IPC Salmo** to each sample well used in the analysis.
3. Create the following real-time PCR program for the analysis:

| | Step repetitions | Temperature | Duration |
|---|---|---|---|
| Step 1 | ×1 | 50°C | 2 minutes |
| Step 2 | ×1 | 95°C | 10 minutes |
| Step 3 | ×45 | 95°C | 15 seconds |
| | | 60°C[1] | 1 minute |

[1] Collection of fluorescence data during the 60°C – 1 minute stage.

4. Place the PCR plate, the PCR strips or the capillaries in the thermal cycler and run the real-time PCR.

## Analysis of the results

### Analysis of the raw data

Refer to the recommendations of the thermal cycler manufacturer for the analysis of the raw data.

1. Position the threshold limits separately for each target of the real-time PCR.
2. For each detector, interpret the results according to the sample $C_t$ values obtained as recommended below.

### Validation

The test is validated if the following criteria are met:

| | Salmo detector | IPC Salmo detector | Validation |
|---|---|---|---|
| EPC Salmo | $C_t = C_{t\,QC}$ SALM of **4a - EPC Salmo** ± 3$C_t$[1] | $C_t < 45$ or $C_t > 45$[2] | PCR validated |
| NCS | $C_t > 45$ | $C_t = C_{t\,QC}$ IPC of **5 - IPC Salmo** ± 3$C_t$[3] | Extraction validated |
| NC | $C_t > 45$ | $C_t > 45$ | PCR reagents validated |

[1] Please refer to the values listed in section 2.1 "EPC" of the Certificate of Analysis of the batch used for the test.
[2] The IPC value in the EPC should not be used for test validation.
[3] Refer to the values listed in section 2.2 "IPC" of the Certificate of Analysis of the batch used for the test.

### Interpretation of results

For each sample analyzed, the results should be interpreted as shown below:

| Salmo detector | IPC Salmo detector | Interpretation |
|---|---|---|
| $C_t$ < 45 | $C_t$ < 45 or $C_t$ > 45 | *Salmonella enterica* spp. detected |
| $C_t$ > 45 | $C_t \leq C_t$ IPC of NCS + 3$C_t$[1] | *Salmonella enterica* spp. not detected |
| $C_t$ > 45 | $C_t > C_t$ IPC of NCS + 3$C_t$[1] | Not validated[2] |

[1] Refer to the IPC $C_t$ value obtained for the NCS done during the same extraction series as the samples to be analyzed. The IPC $C_t$ value obtained for this NCS must first be validated as described above.

[2] The sample will be returned as not validated due to the non-compliant IPC value.

#### Action to be taken for non-validated samples

1. Dilute the sample DNA at a 1:10 dilution in 1X TE buffer.
2. Perform a new PCR analysis on 5 µL of this dilution.
3. If the diluted DNA is positive for *Salmonella* or negative for *Salmonella* with a compliant IPC result, the obtained result is then validated.
4. If the diluted DNA is negative for *Salmonella* with a non-compliant IPC result, the obtained result is still not validated. In this case, repeat the nucleic acid extraction using a sample pre-diluted at 1:10 in 1X PBS buffer before extraction.
5. If the result is still not validated, repeat the analysis on a new sample.

## Documentation and support

### Customer and technical support

Technical support: visit **thermofisher.com/askaquestion**

Visit **thermofisher.com/support** for the latest in services and support, including:

- Worldwide contact telephone numbers
- Order and web support
- User guides, manuals, and protocols
- Certificates of Analysis
- Safety Data Sheets (SDSs; also known as MSDSs)

  **NOTE:** For SDSs for reagents and chemicals from other manufacturers, contact the manufacturer.

### Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale found on Life Technologies' website at **www.thermofisher.com/us/en/home/global/terms-and-conditions.html**. If you have any questions, please contact Life Technologies at **thermofisher.com/support**.

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**Revision history of Pub. No.** MAN0008868 (English)

| Revision | Date | Description |
|---|---|---|
| B.0 | 23 May 2017 | Updated to the current document template, with associated updates to the warranty, trademarks, and logos. |
| A.0 | 20 February 2014 | Baseline for revision history |