



PATHOGEN ANALYSIS THROUGH  
HIGH-QUALITY QPCR

INSTRUCTION MANUAL

**PATHq *Serratia marcescens***  
**Real-Time PCR Kit**  
**PATHq-SMA-001**



# Contents

|                                                  |    |
|--------------------------------------------------|----|
| 1. INTENDED USE .....                            | 03 |
| 2. SUMMARY AND EXPLANATION OF THE TEST .....     | 03 |
| 3. PRINCIPLE OF THE ASSAY .....                  | 03 |
| 4. KIT CONTENTS .....                            | 04 |
| 5. STORAGE AND STABILITY .....                   | 04 |
| 6. MATERIALS REQUIRED BUT NOT SUPPLIED .....     | 04 |
| 7. SAMPLE PREPARATION .....                      | 05 |
| 7.1. Guidelines for DNA Extraction               |    |
| 7.2. Controls                                    |    |
| 8. PCR REACTION SETUP .....                      | 06 |
| 8.1. Reaction Mix (per reaction)                 |    |
| 9. PCR AMPLIFICATION PROTOCOL .....              | 06 |
| 9.1. Standard Cycling Conditions                 |    |
| 9.2. Fast Cycling Conditions                     |    |
| 10. RUN VALIDITY CRITERIA .....                  | 07 |
| 11. INTERPRETATION OF RESULTS .....              | 07 |
| 12. RETESTING SAMPLES WITH INVALID RESULTS ..... | 08 |
| 13. GOOD LABORATORY PRACTICES FOR PCR .....      | 08 |
| 14. LIMITATIONS OF THE ASSAY .....               | 09 |
| 15. TECHNICAL SUPPORT .....                      | 09 |
| 16. VERSION HISTORY .....                        | 09 |

## 1. Intended Use

The PATHq *Serratia marcescens* Real-Time PCR Kit is intended for the qualitative detection of *Serratia marcescens* DNA by Real-Time PCR following nucleic acid extraction from biological samples.

The assay includes an Internal Positive Control (IPC) to monitor nucleic acid extraction efficiency and detect potential PCR inhibition.

The assay can be applied to nucleic acids extracted from bacterial cultures, porcine semen and environmental samples associated with artificial insemination facilities (e.g. water systems and surface swabs).

This product is intended for veterinary laboratory use for research purposes (RUO).

Results obtained with this assay should be interpreted in conjunction with other laboratory findings and relevant epidemiological information.

## 2. Summary and Explanation of the Test

*Serratia marcescens* is a Gram-negative bacterium belonging to the Enterobacteriaceae family. It is widely distributed in the environment and may act as an opportunistic pathogen in animals and humans.

Real-Time PCR provides a rapid and sensitive method for the detection of bacterial DNA. The PATHq *Serratia marcescens* Real-Time PCR Kit enables the amplification and detection of a specific genomic target of *Serratia marcescens* using fluorescent probe technology.

During amplification, the target DNA is detected in the FAM fluorescence channel, while the Internal Positive Control (IPC) is detected in the VIC fluorescence channel, allowing monitoring of PCR performance and detection of potential inhibition.

## 3. Principle of the Assay

After nucleic acid extraction, DNA from the sample is added to a PCR reaction mixture containing sequence-specific primers and probes targeting *Serratia marcescens*.

During amplification, the probe hybridizes to the target sequence and is cleaved by the DNA polymerase, generating a fluorescent signal that increases proportionally to the amount of amplified DNA. Fluorescence is measured in real time by the PCR instrument.

The assay simultaneously detects the target DNA and an exogenous Internal Positive Control (IPC) used to monitor nucleic acid extraction efficiency and PCR inhibition.

The assay detects two targets:

| TARGET                           | REPORTER | QUENCHER |
|----------------------------------|----------|----------|
| <i>Serratia marcescens</i> .     | FAM      | NFQ-MGB  |
| Internal Positive Control (IPC). | VIC      | NFQ-MGB  |

\* ROX must be selected as the passive reference dye.

## 4. Kit Contents

The kit contains reagents sufficient for 100 reactions.

| COMPONENT                                             | Reference    | VOLUME     | DESCRIPTION                         |
|-------------------------------------------------------|--------------|------------|-------------------------------------|
| PATHq PCR Master Mix - <i>Serratia marcescens</i>     | MM-SMA-001   | 2 × 550 µL | Real-Time PCR master mix.           |
| PATHq Primer / Probe Mix - <i>Serratia marcescens</i> | PP-SMA-001   | 110 µL     | Target-specific primers and probe.  |
| PATHq Internal Positive Control - Xeno                | IPC-XENO-001 | 200 µL     | Exogenous Internal Positive Control |
| PATHq Positive Control - <i>Serratia marcescens</i>   | PC-SMA-001   | 100 µL     | Positive amplification control.     |
| PATHq Nuclease - Free Water.                          | WATER-001    | 1000 µL    | PCR-grade water for reaction setup. |

## 5. Storage and Stability

Store all reagents at  $-20 \pm 5$  °C.

Avoid repeated freeze-thaw cycles. Thaw reagents completely and mix thoroughly before use.

When stored under the recommended conditions, the reagents are stable until the expiration date indicated on the kit label.

Protect the primer/probe mix from prolonged exposure to light.

## 6. Materials Required but Not Supplied

- Real-Time PCR instrument compatible with FAM and VIC detection channels
- Nucleic acid extraction kit suitable for the sample type
- PCR tubes or PCR plates
- Aerosol-resistant filter tips
- Nuclease-free microtubes for reagent preparation
- Micropipettes
- Vortex mixer
- Centrifuge for PCR plates/tubes

## 7. Sample Preparation

Nucleic acids should be extracted using validated extraction methods according to the laboratory workflow. Magnetic bead-based extraction systems are recommended.

Addition of Internal Positive Control (IPC): Add 2 µL of IPC per extraction to each sample and to the Negative Extraction Control (NEC) during nucleic acid extraction to monitor extraction efficiency and PCR inhibition.

The IPC should be added before the lysis step of the extraction procedure.

### 7.1. GUIDELINES FOR DNA EXTRACTION

| PARAMETER                                         | RECOMMENDATION                                                                                                                |
|---------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------|
| DNA extraction method.                            | Validated nucleic acid extraction methods should be used. Magnetic bead-based systems are recommended.                        |
| Addition of Internal Positive Control (IPC).      | Add 2 µL of IPC per extraction to each sample and to the Negative Extraction Control (NEC).                                   |
| Preparation of Negative Extraction Control (NEC). | Include at least one Negative Extraction Control (NEC) in each extraction run using nuclease-free water as starting material. |
| Processing of controls.                           | Process the NEC using the same extraction procedure as used for test samples.                                                 |

### 7.2. CONTROLS

The following control must be included in each nucleic acid extraction run:

Negative Extraction Control (NEC): The NEC is prepared using nuclease-free water as starting material and processed together with the samples during nucleic acid extraction. This control monitors potential contamination introduced during sample preparation and extraction procedures.

The Internal Positive Control (IPC) must be added to each sample and to the NEC during nucleic acid extraction to monitor extraction efficiency and PCR inhibition.

| CONTROL                            | PURPOSE                                                    | STAGE            |
|------------------------------------|------------------------------------------------------------|------------------|
| PC - Positive PCR Control.         | Confirms correct PCR amplification of the target sequence. | PCR              |
| NEC - Negative Extraction Control. | Detects contamination during nucleic acid extraction.      | Extraction       |
| NTC - No Template Control.         | Detects contamination in PCR reagents or reaction setup.   | PCR              |
| IPC - Internal Positive Control.   | Monitors extraction efficiency and PCR inhibition.         | Extraction + PCR |

## 8. PCR Reaction Setup

### 8.1. REACTION MIX (PER REACTION)

Prepare the PCR mix according to the following table.

| COMPONENT                                             | VOLUME |
|-------------------------------------------------------|--------|
| PATHq PCR Master Mix - <i>Serratia marcescens</i>     | 10 µL  |
| PATHq Primer / Probe Mix - <i>Serratia marcescens</i> | 1 µL   |
| PATHq Nuclease - Free Water                           | 1 µL   |
| Template / PC / NTC / NEC                             | 8 µL   |

Total reaction volume: 20 µL

Prepare sufficient reaction mix for the total number of reactions, including test samples and controls. It is recommended to prepare an additional 10% excess volume to compensate for pipetting losses.

Prepare the reaction mix by combining the following reagents:

- MM-SMA-001: PATHq PCR Master Mix - *Serratia marcescens*
- PP-SMA-001: PATHq Primer / Probe Mix - *Serratia marcescens*
- WATER-001: PATHq Nuclease - Free Water

Dispense 12 µL of the reaction mix into each PCR tube or well.

Then add 8 µL of one of the following:

- Template (test sample)
- PC-SMA-001: PATHq Positive Control - *Serratia marcescens* (PC)
- WATER-001: PATHq Nuclease - Free Water (NTC)

Do not mix reagents from different kit lots.

## 9. PCR Amplification Protocol

### 9.1. STANDARD CYCLING CONDITIONS

| COMPONENT             | TEMPERATURE | TIME   | CYCLES |
|-----------------------|-------------|--------|--------|
| Initial denaturation. | 95 °C       | 10 min | 1      |
| Denaturation.         | 95 °C       | 15 s   | 40     |
| Annealing/Extension*. | 60 °C       | 60 s   |        |

\* Fluorescence acquisition at 60 °C

## 9.2. FAST CYCLING CONDITIONS

| COMPONENT             | TEMPERATURE | TIME   | CYCLES |
|-----------------------|-------------|--------|--------|
| Initial denaturation. | 95 °C       | 10 min | 1      |
| Denaturation.         | 95 °C       | 3 s    | 40     |
| Annealing/Extension*. | 60 °C       | 30 s   |        |

\* Fluorescence acquisition at 60 °C

## 10. Run Validity Criteria

The PCR run is considered valid when the following criteria are fulfilled:

| CONTROL   | EXPECTED RESULT                 |
|-----------|---------------------------------|
| PC - FAM  | Ct within QC Ct $\pm$ 3 cycles. |
| NTC - FAM | Ct>40 or undetermined           |
| IPC - VIC | Ct $\leq$ 40                    |
| NEC - FAM | Ct>40 or undetermined           |

The expected Ct value of the Positive Control (PC) is defined in the Quality Control documentation (CoA / Certificate of Analysis) provided with each kit lot.

If any of these criteria are not met, the run should be considered invalid and repeated.

## 11. Interpretation of Results

**IMPORTANT:** The Internal Positive Control (IPC) is added during nucleic acid extraction and is therefore expected to amplify in samples and in the NEC, but not in the NTC or EPC reactions.

| SERRATIA (FAM)                     | IPC (VIC)               | INTERPRETATION                             |
|------------------------------------|-------------------------|--------------------------------------------|
| Ct $\leq$ 40                       | $\pm$                   | <i>Serratia marcescens</i> detected.       |
| Ct > 40 or undetermined.           | Ct $\leq$ 40            | <i>Serratia marcescens</i> not detected.   |
| Ct > 40 or undetermined.           | Ct > 40 or undetermined | Invalid result - PCR inhibition suspected. |
| Ct $\leq$ 40 in NTC (FAM channel). |                         | Run invalid - contamination suspected.     |
| No signal in PC.                   | —                       | Run invalid.                               |

**NOTE:** In strongly positive samples, amplification of the IPC may be reduced or absent due to competition during PCR amplification.

## 12. Retesting Samples with Invalid Results

If a sample produces an invalid result:

1. Dilute the extracted DNA 1:10 in nuclease-free water or TE buffer.
2. Repeat the Real-Time PCR using 8 µL of diluted DNA.

Interpret the results as follows:

| RESULT                                                               | INTERPRETATION         |
|----------------------------------------------------------------------|------------------------|
| Serratia detected (FAM).                                             | Sample positive.       |
| Serratia not detected and IPC valid ( $C_t \leq 40$ ).               | Sample negative.       |
| Serratia not detected and IPC invalid ( $C_t > 40$ or undetermined). | Repeat DNA extraction. |

If inhibition persists, repeat nucleic acid extraction from the original sample.

## 13. Good Laboratory Practices for PCR

- Wear appropriate personal protective equipment, including laboratory coat and gloves.
- Use separate laboratory areas and dedicated equipment for:
  - Sample preparation
  - PCR reaction setup
  - Amplification and analysis
- Change gloves frequently.
- Use aerosol-resistant filter tips.
- Avoid opening PCR tubes after amplification to prevent contamination.
- Keep reaction tubes closed whenever possible.
- Clean work surfaces and equipment regularly using 10% bleach or DNA decontamination solutions.

## 14. Limitations of the Assay

- The performance of the assay depends on proper sample collection, handling and nucleic acid extraction.
- The presence of PCR inhibitors in the sample may affect amplification efficiency and lead to false negative results.
- Mutations or sequence variations in the target region may affect detection.
- Results should be interpreted in conjunction with clinical signs, epidemiological data and other laboratory findings.

## 15. Technical Support

OVISLab S.L.

Avenida Comas y Masferrer, 31. Apartado de Correos 40 - 08450 Llinars del Vallés

T. 93.841.11.11 - F. 93.841.23.35

ovislab@ovislab.com - www.ovislab.com

For technical assistance contact: pathqsupport@ovislab.com

## 16. Version History

| VERSION | DATE    | DESCRIPTION                                                                                                                                                               |
|---------|---------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| V01     | 08-2026 | Initial release                                                                                                                                                           |
| V02     | 09-2026 | <ul style="list-style-type: none"><li>• Added ROX as passive reference dye.</li><li>• Added QUENCHER column to the target table.</li><li>• Redesigned Table 12.</li></ul> |

## Instruction manual PATHq *Serratia marcescens* Real-Time PCR Kit

OVISLab S.L.

Avenida Comas y Masferrer, 31. Apartado de Correos 40 - 08450 Llinars del Vallés

T. 93.841.11.11 - F. 93.841.23.35

ovislab@ovislab.com - [www.ovislab.com](http://www.ovislab.com)

For technical assistance contact: [pathqsupport@ovislab.com](mailto:pathqsupport@ovislab.com)

