IVD For *in Vitro* Diagnostic Use

CE

# Pseudomonas aeruginosa Real-TM Quant

# Handbook

## Real Time PCR Kit for quantitative detection of Pseudomonas aeruginosa

**REF B76-50FRT**

**Σ 50**

## NAME

**Pseudomonas aeruginosa Real-TM Quant**

## INTRODUCTION

*Pseudomonas* is a gram-negative rod that belongs to the family Pseudomonadaceae. These Pseudomonas aeruginosas are widespread in nature, inhabiting soil, water, plants, and animals (including humans). *Pseudomonas aeruginosa* has become an important cause of infection, especially in patients with compromised host defense mechanisms.

## INTENDED USE

**Pseudomonas aeruginosa Real-TM Quant** kit is a Real-Time test for the Qualitative and Quantitative detection of *Pseudomonas aeruginosa*.

## PRINCIPLE OF ASSAY

**Pseudomonas aeruginosa Real-TM Quant** kit is a Real-Time test for the Qualitative and Quantitative detection of *Pseudomonas aeruginosa* in the biological materials (sputum, aspirate from trachea, nasopharyngeal swabs, throat swabs, bronchoalveolar lavage, tissue) and in environmental samples (water, washes from environmental objects, biofilms scrapes, ground). DNA is extracted from samples, amplified and detected using fluorescent reporter dye probes specific for *Pseudomonas aeruginosa* DN and Internal Control IC. Internal Control (IC), added during the sample preparation serves as an amplification control for each individually processed specimen and to identify possible reaction inhibition.

*Pseudomonas aeruginosa* DNA amplification is detected on JOE(Yellow)/HEX/Cy3 channel, the IC DNA amplification is detected on FAM (Green) channel.

Quantitative DNA analysis is based on the linear dependence between the cycle threshold (Ct) and the initial concentration of DNA target. Quantitative analysis is performed in the presence of DNA calibrators (samples with a known concentration of *Pseudomonas aeruginosa* DNA), which are added during amplification. The results of amplification of DNA calibrators are used to construct a calibration curve, on the basis of which the concentration of *Pseudomonas aeruginosa* DNA in samples determined. To minimize the effect of variation during material sampling, the quantitative results (*Pseudomonas aeruginosa* DNA concentrations) are normalized to the genomic DNA quantity.

## MATERIALS PROVIDED

### Module No.1: Real Time PCR kit (B76-50FRT)

- **PCR-mix-1**, 0,6 ml
- **PCR-mix2- FRT**, 0,3 ml
- **TaqF DNA Polymerase**, 0,03 ml
- **TE-buffer**, 0,5 ml
- **Negative Control C-***, 1,2 ml
- **Pos C+** *(P.aeruginosa* DNA) ***, 0,1 ml
- **Internal Control IC****, 0,6 ml
- **Standard *P. aeruginosa* DNA/IC**
  - **QSG1**, 0,1 ml
  - **QSG2**, 0,1 ml

Contains reagents for 55 tests.

** must be used during the sample preparation procedure: add 100 µl of C– (Negative Control) to labeled Cneg;*
***add 10 µl of Internal Control to all samples during the DNA isolation procedure directly to the sample/lysis mixture*
**** must be used in the extraction procedure as Positive Control of Extraction. Add 10 µl of Pos C+ and 90 µl of C- to the tube labeled Cpos.*

## MATERIALS REQUIRED BUT NOT PROVIDED

- DNA isolation kit
- Desktop microcentrifuge for “eppendorf” type tubes
- Vortex mixer
- Disposable gloves, powderless
- Biohazard waste container
- Refrigerator, Freezer
- Real Time Thermal cycler
- Workstation
- Pipettes (adjustable)
- Sterile pipette tips with filters

## STORAGE INSTRUCTIONS

**Pseudomonas aeruginosa Real-TM Quant** must be stored at -20°C. The **Pseudomonas aeruginosa Real-TM Quant** kit can be shipped at 2-8°C but should be immediately stored at -20°C on receipt.

## STABILITY

**Pseudomonas aeruginosa Real-TM Quant** Test is stable up to the expiration date indicated on the kit label. The product will maintain performance through the control date printed on the label. Exposure to light, heat or humidity may affect the shelf life of some of the kit components and should be avoided. Repeated thawing and freezing of these reagents should be avoided, as this may reduce the sensitivity.

## QUALITY CONTROL

In accordance with Sacace's ISO 13485-Certified Quality Management System, each lot is tested against predetermined specifications to ensure consistent product quality.

## WARNINGS AND PRECAUTIONS

IVD

***In Vitro* Diagnostic Medical Device**

For *In Vitro* Diagnostic Use Only

The user should always pay attention to the following:

- Use sterile pipette tips with aerosol barriers and use new tip for every procedure.
- Store extracted positive material (samples, controls and amplicons) away from all other reagents and add it to the reaction mix in a separate area.
- Thaw all components thoroughly at room temperature before starting an assay.
- When thawed, mix the components and centrifuge briefly.
- Use disposable gloves, laboratory coats and eye protection when handling specimens and reagents. Thoroughly wash hands afterwards.
- Do not eat, drink, smoke, apply cosmetics, or handle contact lenses in laboratory work areas.
- Do not use a kit after its expiration date.
- Dispose of all specimens and unused reagents in accordance with local authorities' regulations.
- Specimens should be considered potentially infectious and handled in a biological cabinet in accordance with appropriate biosafety practices.
- Clean and disinfect all sample or reagent spills using a disinfectant such as 0.5% sodium hypochlorite, or other suitable disinfectant.
- Avoid sample or reagent contact with the skin, eyes, and mucous membranes. If skin, eyes, or mucous membranes come into contact, rinse immediately with water and seek medical advice immediately.
- Material Safety Data Sheets (MSDS) are available on request.
- Use of this product should be limited to personnel trained in the techniques of DNA amplification.
- The laboratory process must be one-directional, it should begin in the Extraction Area and then move to the Amplification and Detection Areas. Do not return samples, equipment and reagents to the area in which the previous step was performed.

Some components of this kit contain sodium azide as a preservative. Do not use metal tubing for reagent transfer.

## PRODUCT USE LIMITATIONS

All reagents may exclusively be used in in vitro diagnostics. Use of this product should be limited to personnel trained in the techniques of DNA amplification (UNI EN ISO 18113-2:2012). Strict compliance with the user manual is required for optimal PCR results. Attention should be paid to expiration dates printed on the box and labels of all components. Do not use a kit after its expiration date.

## SAMPLE COLLECTION, STORAGE AND TRANSPORT

**Pseudomonas aeruginosa Real-TM Quant** can analyze DNA extracted from:

- *sputum*
- *nasopharyngeal*
- *bronchoalveolar lavage*
- *swabs*
- *plasma*

Specimens can be stored at +2-8°C for no longer than 12 hours, or freeze at -20°C to -80°C.Transportation of clinical specimens must comply with country, federal, state and local regulations for the transport of etiologic agents.

## DNA ISOLATION

Any commercial RNA/DNA isolation kit, if IVD-CE validated for the specimen types indicated herein at the “SAMPLE COLLECTION, STORAGE AND TRANSPORT” paragraph, could be used.

Sacace Biotechnologies recommends to use the following kit:

- ⇨ **DNA/RNA-Prep** (Sacace, REF K-2-9);
- ⇨ **SaMag Bacterial DNA Extraction kit** (Sacace, REF SM006);

Please carry out DNA extraction according to the manufacture’s instruction.

Add 10 µl of Internal Control during DNA isolation procedure directly to the sample/lysis mixture.

## PROTOCOL:

1. Prepare required quantity of tubes or PCR plate.
2. Prepare for each sample in the new sterile tube **10*N µl** of **PCR-mix-1**, **5*N µl** of **PCR-mix-2-FRT** and **0,5*N µl** of **Hot Start DNA Polymerase.**
3. Add **15 µl** of **Reaction Mix** into each tube.
4. Add **10 µl** of **extracted DNA** sample to appropriate tube with Reaction Mix.
5. Prepare for qualitative run 1 positive control and 1 negative control:
   - add **10 µl** of **QSG2** to the tube labeled C*pos*;
   - add **10 µl** of **TE-buffer** to the tube labeled C*neg*;
6. For quantitative analysis prepare 4 tubes and perform QSG1 and QSG2* standards twice.
   **QSG1 and QSG2 values are specific for each lot and are reported in the Quant Data Card provided in the kit.*

Close tubes and transfer them into the instrument in this order: samples, negative controls, positive control, Standards.

### Amplification

1. Create a temperature profile on your instrument as follows:

| | Rotor-type Instruments[1] | | | Plate- or modular type Instruments[2] | | |
|---|---|---|---|---|---|---|
| **Step** | ***Temperature, °C*** | ***Time*** | **Repeats** | ***Temperature, °C*** | ***Time*** | **Repeats** |
| 1 | **95** | 15 min | 1 | **95** | 15 min | 1 |
| 2 | **95** | 5 s | 5 | **95** | 5 s | 5 |
| | **60** | 20 s | | **60** | 20 s | |
| | **72** | 15 s | | **72** | 15 s | |
| 3 | **95** | 5 s | 40 | **95** | 5 s | 40 |
| | **60** | 20 s *fluorescent signal detection* | | **60** | 30 s *fluorescent signal detection* | |
| | **72** | 15 s | | **72** | 15 s | |

[1] *For example Rotor-Gene™ 3000/6000/Q (Corbett Research, Qiagen)*
[2] *For example, SaCycler-96™ (Sacace), CFX/iQ5™ (BioRad); Mx3005P™ (Agilent), ABI® 7300/7500/StepOne Real Time PCR (Applied Biosystems), SmartCycler® (Cepheid), LineGeneK® (Bioer)*

Fluorescence is detected at the 2nd phase of Step 3 (60 °C) in FAM/Green and JOE/Yellow/Hex/Cy3 fluorescence channels.

*Pseudomonas aeruginosa* DNA amplification is detected on JOE(Yellow)/HEX/Cy3 channel, IC DNA amplification is detected on FAM (Green) channel.

## INSTRUMENT SETTINGS

**Rotor-type instruments (RotorGene 3000/6000, RotorGene Q)**

| Channel | Threshold | More Settings/ Outlier Removal | Slope Correct | Calibrate/Gain Optimization |
|---|---|---|---|---|
| FAM/Green | 0.03 | 10 % | on | 5Fl-10Fl |
| JOE/Yellow | 0.03 | 10 % | on | 5Fl-10Fl |

**Plate- or modular type instruments**

The threshold line should cross only sigmoid curves of signal accumulation of positive samples and should not cross the baseline; otherwise, the threshold level should be raised. Set the threshold at a level where fluorescence curves are linear and do not cross curves of the negative samples.

## RESULTS INTERPRETATION

The results are interpreted through the presence of crossing of fluorescence curve with the threshold line. To set threshold put the line at such level where curves of fluorescence are linear.

- *Pseudomonas aeruginosa* DNA amplification is detected on JOE(Yellow)/HEX/Cy3 channel;
- IC DNA amplification is detected on FAM (Green) channel.

### Qualitative analysis

Results are accepted as relevant if positive and negative controls of amplification and extraction are passed.

**Results for controls**

| Control | Stage for control | Ct FAM (Green) | Ct JOE(Yellow)/HEX/Cy3 | Interpretation |
|---|---|---|---|---|
| **NCE** | DNA isolation | Pos | - | OK |
| **Pos C+** | DNA isolation | Pos | Pos | OK |
| **NCA** | PCR | _ | _ | OK |
| **QSG2** | PCR | Pos | Pos | OK |

- The sample is considered to be positive for *Pseudomonas aeruginosa* if in the channel JOE(Yellow)/HEX/Cy3 the value of **Ct** is different from zero (Ct<35);
- The sample is considered to be uncertain for *Pseudomonas aeruginosa* if its Ct value is more than 35 on JOE(Yellow)/HEX/Cy3 channel. Additional double study of this sample should be conducted;
- Specimens with Ct < 30 in the channel FAM (Green) and absent fluorescence signal in the channel JOE(Yellow)/HEX/Cy3 are interpreted as negative.

### Quantitative analysis

For each control and patient specimen, calculate the concentration of *Pseudomonas aeruginosa* DNA in 1 ml of sample using following formula:

$$\frac{\textbf{\textit{P. aeruginosa} DNA copies/specimen (JOE(Yellow)/HEX/Cy3 channel)}}{\textbf{IC DNA copies/specimen (Fam(Green) channel)}} \textbf{ x IC coefficient* = copies \textit{P. aeruginosa} /ml}$$

**coefficient is specific for each lot and reported in the **P. aeruginosa** Quant Data Card provided in the kit.*

## PERFORMANCE CHARACTERISTICS

### Analytical specificity

The analytical specificity of the primers and probes was validated with negative samples. They did not generate any signal with the specific *Pseudomonas aeruginosa* primers and probes. The specificity of the kit **Pseudomonas aeruginosa Real-TM Quant** was 100%. The potential cross-reactivity of the kit **Pseudomonas aeruginosa Real-TM Quant** was tested against the group control (*Streptococcus pyogenes, Staphylococcus aureus, Streptococcus agalactiae, Neisseria meningitidis, Streptococcus pneumoniae, Haemophilus influenzae, Listeria monocitogenes, Proteus vulgaris, Enterobacter faecalis* and other ones). It was not observed any cross-reactivity with other Pseudomonas aeruginosas.

### Analytical sensitivity

The kit **Pseudomonas aeruginosa Real-TM Quant** allows to detect *Pseudomonas aeruginosa* DNA in 100% of the tests with a sensitivity of not less than 500 copies/ml.

## LINEARITY

**Pseudomonas aeruginosa Real-TM Quant** is linear from 800 to 1 x $10^7$ copies/ml.

## QUALITY CONTROL PROCEDURE

A defined quantity of Internal Control (IC) is introduced into each sample and control at the beginning of sample preparation procedure in order to control the extraction process of each individual sample and to identify possible reaction inhibition.

A negative control of extraction (NCE), negative amplification control (NCA), positive amplification control (C+) are required for every run to verify that the specimen preparation, the amplification and the detection steps are performed correctly.

If the controls are out of their expected range (see table Results for Controls), all of the specimens and controls from that run must be processed beginning from the sample preparation step.

## TROUBLESHOOTING

1. Weak or no signal of the IC (Fam/channel).
    - The PCR was inhibited.
        ⇒ Make sure that you use a recommended DNA extraction method and follow to the manufacturer's instructions.
    - The reagents storage conditions didn't comply with the instructions.
        ⇒ Check the storage conditions
    - The PCR conditions didn't comply with the instructions.
        ⇒ Check the PCR conditions and select for the IC detection the fluorescence channel reported in the protocol.
2. Weak or no signal of the Positive Control.
    - The PCR conditions didn't comply with the instructions.
        ⇒ Check the amplification protocol and select the fluorescence channel reported in the manual.
3. JOE(Yellow)/HEX/Cy3 signal with Negative Control of extraction.
    - Contamination during DNA extraction procedure. All samples results are invalid.
        ⇒ Decontaminate all surfaces and instruments with sodium hypochlorite and ethanol.
        ⇒ Use only filter tips during the extraction procedure. Change tips between tubes.
        ⇒ Repeat the DNA extraction with the new set of reagents.
4. Any signal with Negative Control of PCR (DNA-buffer).
    - Contamination during PCR preparation procedure. All samples results are invalid.
        ⇒ Decontaminate all surfaces and instruments with sodium hypochlorite and ethanol or special DNA decontamination reagents.
        ⇒ Repeat the PCR preparation with the new set of reagents.

## KEY TO SYMBOLS USED

| | | | |
|---|---|---|---|
| REF | List Number |  | Caution! |
| LOT | Lot Number | | Contains sufficient for <n> tests |
| IVD | For *in Vitro* Diagnostic Use | VER | Version |
|  | Store at | **NCA** | Negative Control of Amplification |
|  | Manufacturer | **C–** | Negative control of Extraction |
|  | Consult instructions for use | **C+** | Positive Control of Amplification |
|  | Expiration Date | **IC** | Internal Control |




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