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For Professional Use Only

# AmpliSens® *Pseudomonas aeruginosa*-screen-titre-FRT

PCR kit

## Instruction Manual

**AmpliSens®**

EC REP

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**TABLE OF CONTENTS**

## 1. INTENDED USE

**AmpliSens® *Pseudomonas aeruginosa*-screen-titre-FRT** PCR kit is an *in vitro* nucleic acid amplification test for qualitative detection and quantitation of the DNA of *Pseudomonas aeruginosa* in the clinical material (blood, blood plasma, oropharyngeal swab, bronchoalveolar lavage, sputum, endotracheal aspirate, urine, prostatic fluid, cerebrospinal fluid, punctate from the lesions of organs and tissues) by using real-time hybridization-fluorescence detection of amplified products.

The results of PCR analysis are taken into account in complex diagnostics of disease.

## 2. PRINCIPLE OF PCR DETECTION

*Pseudomonas aeruginosa* detection by the polymerase chain reaction (PCR) is based on the amplification of the pathogen genome specific region using specific primers. In the real-time PCR, the amplified product is detected with the use of fluorescent dyes. These dyes are linked to oligonucleotide probes, which bind specifically to the amplified product during thermocycling. The real-time monitoring of fluorescence intensities during the real-time PCR allows the detection of accumulating product without re-opening the reaction tubes after the PCR run.

**AmpliSens® *Pseudomonas aeruginosa*-screen-titre-FRT** PCR kit is a qualitative test that contains the Internal Control (Internal Control STI-87). It must be used in the extraction procedure in order to control the extraction process of each individual sample.

**AmpliSens® *Pseudomonas aeruginosa*-screen-titre-FRT** PCR kit uses “hot-start”, which greatly reduces the frequency of nonspecifically primed reactions. “Hot-start” is guaranteed by the separation of nucleotides and Taq-polymerase by using chemically modified polymerase (TaqF). The chemically modified polymerase (TaqF) is activated by heating at 95 °C for 15 min.

## 3. CONTENT

**AmpliSens® *Pseudomonas aeruginosa*-screen-titre-FRT** PCR kit is produced in 1 form:
**AmpliSens® *Pseudomonas aeruginosa*-screen-titre-FRT** PCR kit variant FRT-50 F, **REF** R-B76-50-FT(RG,iQ)-CE

**AmpliSens® *Pseudomonas aeruginosa*-screen-titre-FRT PCR kit variant FRT-50 F** includes:

| ***Reagent*** | ***Description*** | ***Volume, ml*** | ***Quantity*** |
|---|---|---|---|
| **PCR-mix-1-FRT *P.aeruginosa*** | colorless clear liquid | 0.6 | 1 tube |

| | | | |
|---|---|---|---|
| **PCR-mix-2-FRT** | colorless clear liquid | 0.3 | 1 tube |
| **Polymerase (TaqF)** | colorless clear liquid | 0.03 | 1 tube |
| **DNA-buffer** | colorless clear liquid | 0.5 | 1 tube |
| **DNA calibrator C1 *P.aeruginosa*** | colorless clear liquid | 0.1 | 1 tube |
| **DNA calibrator C2 *P.aeruginosa*** | colorless clear liquid | 0.1 | 1 tube |
| **Negative Control (C–)*** | colorless clear liquid | 1.2 | 1 tube |
| **Internal Control STI-87 (IC)**** | colorless clear liquid | 0.6 | 1 tube |
| **Positive control DNA *P.aeruginosa****** | colorless clear liquid | 0.1 | 1 tube |

* must be used in the extraction procedure as Negative Control of Extraction.

** add 10 µl of Internal Control during the DNA extraction procedure directly to the sample/lysis mixture (RIBO-prep, **REF** K2-9-Et-50-CE protocol).

*** must be used in the extraction procedure as Positive Control of Extraction.

**AmpliSens®** ***Pseudomonas aeruginosa*-screen-titre-FRT** PCR kit is intended for 60 reactions (including controls).

## 4. ADDITIONAL REQUIREMENTS

- DNA extraction kit or the DNA extraction automatic station.
- Disposable powder-free gloves and a laboratory coat.
- Pipettes (adjustable).
- Sterile pipette tips with aerosol filters (100 and 200 µl).
- Tube racks.
- Vortex mixer.
- PCR box.
- Real-time instruments (for example, Rotor-Gene 3000/6000 (Corbett Research, Australia); Rotor-Gene Q (QIAGEN, Germany); iCycler iQ5 (Bio-Rad, USA); Mx3000P (Stratagene, USA)).
- Disposable polypropylene PCR tubes (0.1- or 0.2-ml):
    a) 0.2-ml PCR tubes with optical transparent domed or flat caps if a plate-type instrument is used;
    b) 0.2-ml PCR tubes with flat caps or strips of four 0.1-ml Rotor-Gene PCR tubes if a rotor-type instrument is used.
- Refrigerator with the range from 2 to 8 °C.
- Deep-freezer with the range from minus 24 to minus 16 °C.

- Reservoir for used tips.

## 5. GENERAL PRECAUTIONS

The user should always pay attention to the following:

- Use sterile pipette tips with aerosol filters and use a new tip for every procedure.
- Store all extracted positive material (samples, controls and amplicons) away from all other reagents and add it to the reaction mix in a distantly separated facility.
- Thaw all components thoroughly at room temperature before starting an assay.
- When thawed, mix the components and centrifuge briefly.
- Use disposable protective gloves and laboratory cloths, and protect eyes while samples and reagents handling. Thoroughly wash hands afterward.
- 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 samples and unused reagents in accordance with local regulations.
- Samples should be considered potentially infectious and handled in a biological cabinet in accordance with appropriate biosafety practices.
- Clean and disinfect all samples or reagents spills using a disinfectant, such as 0.5 % sodium hypochlorite or another suitable disinfectant.
- Avoid samples and reagents contact with the skin, eyes and mucous membranes. If these solutions come into contact, rinse the injured area 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 DNA amplification techniques.
- Workflow in the laboratory must be one-directional, beginning in the Extraction Area and moving to the Amplification and Detection Area. Do not return samples, equipment, and reagents to the area where the previous step was performed.

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

## 6. SAMPLING AND HANDLING

Obtaining samples of biological materials for PCR-analysis, transportation, and storage are described in the manufacturer's handbook [1]. It is recommended that this handbook is read before starting work.

**AmpliSens®** ***Pseudomonas aeruginosa*-screen-titre-FRT** PCR kit is intended for

analysis of the DNA extracted with DNA extraction kits from the clinical material (blood, blood plasma, oropharyngeal swab, bronchoalveolar lavage, sputum, endotracheal aspirate, urine, prostatic fluid, cerebrospinal fluid, punctate from the lesions of organs and tissues).

*Pretreatment*

6.1 *Blood plasma* is obtained by centrifugation of the tubes with whole blood at 800 g for 10 min at room temperature. Then not less than 1 ml of blood plasma sample is taken with separate tips with aerosol filters into sterile 1.5-2 ml Eppendorf tubes. Centrifuged 1 ml of plasma at 11,000 rpm for 10-20 min. DNA should be extracted from the pellet with 100 µl of supernatant.

## 7. WORKING CONDITIONS

**AmpliSens® *Pseudomonas aeruginosa*-screen-titre-FRT** PCR kit should be used at 18–25 °C.

## 8. PROTOCOL

### 8.1. DNA extraction

It is recommended to use the following nucleic acid extraction kits:

– RIBO-prep, **REF** K2-9-Et-50-CE;

– NucliSENS easyMAG automated system (for details see Guidelines [2]).

Extract the DNA according to the manufacturer's protocol.

If extracting with RIBO-prep reagent kit pay attention to the following:
- DNA is extracted in the presence of the internal control sample: add 10 µl of Internal Control STI-87 (IC) to each sample.
- to the tube labeled C– (Negative Control of extraction) transfer 100 µl of Negative Control (C–) reagent;
- to the tube labeled PCE (Positive Control of extraction) transfer 90 µl of Negative Control (C–) and 10 µl of Positive control DNA *P.aeruginosa*.

### 8.2. Preparing PCR

#### 8.2.1 Preparing tubes for PCR

The total reaction volume is **25 µl**, the volume of the **DNA** sample is **10 µl**.

1. Prepare the mixture of **PCR-mix-2-FRT** and **polymerase (TaqF)**. To do this, transfer the entire content of the tube with **polymerase (TaqF) (30 µl)** into the tube with **PCR-mix-2-FRT (300 µl)** and carefully vortex. Avoid foaming. Indicate the date of mixture preparation on the tube.

The prepared mixture is intended for 60 samples.
Store at 2–8 °C for 3 months and use as needed.

If the mixture volume will not be utilized within 3 months it is necessary to prepare mixture for less number of reactions. For example, mix **150 µl** of **PCR-mix-2-FRT** and **15 µl** of **polymerase (TaqF)**. The obtained mixture is intended for 30 reactions.

2. Prepare the reaction mixture. Note that for analysis of even one clinical sample it is necessary to carry out five controls of amplification stage: two DNA calibrators (**K1 *P.aeruginosa*** and **K2 *P.aeruginosa***) in two repeats and the Negative Control of amplification (DNA-buffer). In addition, include one extra reaction when calculating reagent volumes: for detection of N samples take the reagents for N+1 reactions.
3. Mix **PCR-mix-1-FRT *P.aeruginosa*** and the mixture of **PCR-mix-2-FRT** and **polymerase (TaqF)** in a new tube in the following proportion:
   - **10 µl** of **PCR-mix-1-FRT *P.aeruginosa*,**
   - **5 µl** of the mixture of **PCR-mix-2-FRT** and **polymerase (TaqF).**

One can calculate reagent volume for the needed number of reactions according to the scheme given in the Table 1.

Table 1

**Scheme of reaction mixture preparation for variant FRT-50 F**

| | **Reagent volume for specified number of reactions** | |
|---|---|---|
| **Reagent volume per one reaction, µl** | **10.0** | **5.0** |
| **Number of test samples[1]** | **PCR-mix-1-FRT *P.aeruginosa*** | **PCR-mix-2-FRT and Polymerase (TaqF)** |
| **1** | **70** | **35** |
| **2** | **80** | **40** |
| **3** | **90** | **45** |
| **4** | **100** | **50** |
| **5** | **110** | **55** |
| **6** | **120** | **60** |
| **7** | **130** | **65** |
| **8** | **140** | **70** |
| **9** | **150** | **75** |
| **10** | **160** | **80** |
| **11** | **170** | **85** |
| **12** | **180** | **90** |
| **13** | **190** | **95** |
| **14** | **200** | **100** |

[1] Values are given with account of one extra reaction and five controls of amplification stage: 2 DNA calibrators, K1 *P.aeruginosa* and K2 *P.aeruginosa*, (in two replicates) and the Negative Control (DNA-buffer)

| | | |
|---|---|---|
| **15** | **210** | **105** |
| **16** | **220** | **110** |
| **17** | **230** | **115** |
| **18** | **240** | **120** |
| **19** | **250** | **125** |
| **20** | **260** | **130** |
| **21** | **270** | **135** |
| **22** | **280** | **140** |
| **23** | **290** | **145** |
| **24** | **300** | **150** |
| **25** | **310** | **155** |
| **30** | **360** | **180** |

4. Take the required quantity of tubes for amplification of clinical and control DNA samples.
5. Transfer **15 µl** of the prepared mixture to each tube.
6. Add **10 µl** of **DNA** obtained from clinical or control samples to the tubes with the reaction mixture.
7. Prepare control reaction:

| | |
|---|---|
| **NCA** | - add **10 µl** of **DNA-buffer** to the tube labeled NCA (Negative Control of amplification). |
| **C1 *P.aeruginosa*** **C2 *P.aeruginosa*** | - add **10 µl** of **C1 *P.aeruginosa*** to two tubes and **10 µl** of **C2 *P.aeruginosa*** to the other two tubes. |
| **C–** | - add **10 µl** of the sample extracted from the **Negative Control (C–)** reagent (Negative Control of extraction) |
| **PCE** | - add **10 µl** of **DNA** extracted from the **Positive Control DNA *P.aeruginosa*** (Positive Control of extraction). |

### 8.2.2. Amplification

1. Create a temperature profile on your instrument as follows (see Table 2):

Table 2

**AmpliSens-1 amplification program**

| | ***Rotor-type instruments*[2]** | | | ***Plate-type instruments*[3]** | | |
|---|---|---|---|---|---|---|
| ***Step*** | ***Temperature, °C*** | ***Time*** | ***Cycles*** | ***Temperature, °C*** | ***Time*** | ***Cycles*** |
| 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 Fluorescence acquiring | | 60 | 30 s Fluorescence acquiring | |

[2] For example, Rotor-Gene 3000/Rotor-Gene 6000 (Corbett Research, Australia).
[3] For example, iCycler iQ, iQ5 (Bio-Rad, USA).

| | | | | | | |
|---|---|---|---|---|---|---|
| | 72 | 15 s | | 72 | 15 s | |

Fluorescent signal is detected in the channels for the FAM and JOE fluorophores.

2. Insert tubes into the reaction module of the device.
3. Run the amplification program with fluorescence detection.
4. Analyse results after the amplification program is completed.

## 9. DATA ANALYSIS

Analysis of results is performed by the software of the real-time PCR instrument used by measuring fluorescence signal accumulation in two channels:

- The signal of the Internal Control STI-87 DNA amplification product is detected in the channel for the FAM fluorophore.
- The signal of the *Pseudomonas aeruginosa* DNA amplification product is detected in the channel for the JOE fluorophore.

Results are interpreted by the crossing (or not-crossing) the fluorescence curve with the threshold line set at the specific level that corresponds to the presence (or absence) of a *Ct* value of the cDNA sample in the corresponding column of the results grid.

According to the obtained values of *Ct* (crossing of the fluorescence curve with the threshold line set at the specific level) and the calibrators (K1 *P.aeruginosa* and K2 *P.aeruginosa*) values (specified in *Important Product Information Bulletin*), the device program automatically construct the calibration line and calculated values of the number of copies of Positive Control of Extraction DNA (JOE fluorophore) and Internal Control DNA (FAM fluorophore) in the sample. The obtained values are used to calculate the concentration of ***Pseudomonas aeruginosa*** DNA in the test and control samples according to the formula:

**Calculate of concentration of *Pseudomonas aeruginosa* DNA in 1 ml of the sample extracted from 100 µl:**

$$\frac{\text{Number of copies of } \textit{P.aeruginosa} \text{ DNA in the sample}}{\text{Number of copies IC DNA in the sample}} \times \text{IC coefficient (copies/ml)} = \text{copies/ml}$$

**Calculate of concentration of *Pseudomonas aeruginosa* DNA in 1 ml of the sample extracted from volume over 100 µl:**

$$\frac{\text{Number of copies of } \textit{P.aeruginosa} \text{ DNA in the sample}}{\text{Number of copies IC DNA in the sample}} \times n \times \text{IC coefficient (copies/ml)} = \text{copies/ml}$$

$$n = \frac{100}{\text{extraction volume, µl}}$$

The IC coefficient is specified in the Important Product Information Bulletin for the PCR kit.

Principle of interpretation is the following:

– *Pseudomonas aeruginosa* DNA is **detected** if the *Ct* value determined in the results grid in the channel for the JOE fluorophore and calculated concentration is greater or equal than the specified value. Moreover, the fluorescence curve of the sample should cross the threshold line in the area of typical exponential growth of fluorescence.

– *Pseudomonas aeruginosa* DNA is **not detected** in a sample if the *Ct* value is not determined (absent) (fluorescence curve does not crossing threshold) in the channels for JOE fluorophore or calculated concentration less than the specified value and the *Ct* value determined in the channel for the FAM fluorophore and calculated concentration greater than the specified value.

– The result is **invalid** if the *Ct* value is not determined (absent) in the channel for JOE fluorophore, and the *Ct* value in the channel for the FAM fluorophore is not determined (absent) or calculated concentration less than specified value. In such cases, the PCR analysis should be repeated for corresponding clinical sample.

Linear measuring range of the reagent kit: 800 – 10,000,000 copies/ml. If the result is greater than 10,000,000 copies/ml, it is indicated as ***the result is greater than 10,000,000 copies/ml***. If the result is less than 800 copies/ml, it is indicated as ***the result is less than 800 copies/ml***.

Concentration boundary values of DNA calibrators are specified in *Important Product Information Bulletin*

Boundary *Ct* values are specified in the *Important Product Information Bulletin* enclosed to the PCR kit. See also Guidelines [2]

**The result of the analysis is considered reliable only if the results obtained for Positive and Negative Controls of amplification as well as for the Negative and Positive Control of DNA extraction are correct (seeTable 3).**

Table 3

**Results for controls**

| **Control** | **Stage for control** | **Amplification results in the channel for fluorophore** | |
|---|---|---|---|
| | | **FAM** | **JOE** |
| C– | DNA extraction | **>**boundary value | **<**boundary value |
| PCE | DNA extraction | **>**boundary value | Value is within the specified range |
| NCA | PCR | Absent | Absent |

| | | | |
|---|---|---|---|
| C1 *P.aeruginosa*<br>C2 *P.aeruginosa* | PCR | *Ct* value and calculated concentration are determined | *Ct* value and calculated concentration are determined |

## 10. TROUBLESHOOTING

Results of analysis are not taken into account in the following cases:

1. If an **invalid** result is obtained it is necessary to repeat PCR analysis (beginning with the DNA extraction stage) of the required biological sample.
2. Absence of a positive signal in calibrators may indicate incorrect settings of amplification program and other errors made at PCR preparation stage. In that case it is necessary to repeat PCR again for all samples.
3. If for the Negative Control of extraction (C–) in the channel for the JOE fluorophore defined concentration greater than the specified value or for the Negative Control of amplification (NCA) in the channel for the FAM and/or JOE fluorophore determined the *Ct* value, it means that contamination of reagents or samples has occurred. In that case results for all samples are considered to be invalid. The analysis must be repeated and measures for detecting and eliminating the contamination source must be taken.
4. If a positive result is detected for a test sample, whereas its fluorescent curve does not have exponential slope (it more looks like straight line), it means that the threshold or baseline parameters are set incorrectly. This result can't be considered as positive. If the threshold value was correct it is necessary to repeat PCR for this sample.

## 11. TRANSPORTATION

**AmpliSens® *Pseudomonas aeruginosa*-screen-titre-FRT** PCR kit should be transported at 2–8 °C for no longer than 5 days.

## 12. STABILITY AND STORAGE

All components of the **AmpliSens® *Pseudomonas aeruginosa*-screen-titre-FRT** PCR kit are to be stored at 2–8 °C when not in use (except for PCR-mix-1-FRT *P.aeruginosa*, PCR-mix-2-FRT, and polymerase (TaqF)). All components of the **AmpliSens® *Pseudomonas aeruginosa*-screen-titre-FRT** PCR kit are stable until the expiry date stated on the label. The shelf life of reagents before and after the first use is the same, unless otherwise stated.

PCR-mix-1-FRT *P.aeruginosa,* PCR-mix-2-FRT and polymerase (TaqF) are to be stored at the temperature from minus 24 to minus 16 °C

PCR-mix-1-FRT *P.aeruginosa* is to be kept away from light

## 13. SPECIFICATIONS

### 13.1. Sensitivity

| Clinical material | Nucleic acid extraction kit | PCR kit | Sensitivity, copies/ml | Linear measuring range, copies/ml |
|---|---|---|---|---|
| - blood, blood plasma,<br>- oropharyngeal swab,<br>- bronchoalveolar lavage,<br>- sputum,<br>- endotracheal aspirate,<br>- urine,<br>- prostatic fluid,<br>- cerebrospinal fluid,<br>- punctate from the lesions of organs and tissues | RIBO-prep | PCR kit variant FRT, FRT-50 F | 500 | 800 – 10 000 000 |

### 13.2. Specificity

The analytical specificity of **AmpliSens®** ***Pseudomonas aeruginosa*****-screen-titre-FRT** PCR kit is ensured by the selection of specific primers and probes as well as stringent reaction conditions. The primers and probes have been checked for possible homologies to all sequences published in gene banks by sequence comparison analysis.

The activity of the reagents kit components is absence for the DNA from other bacterial pathogens (*Enterobacter faecalis*, *Escherichia coli*, *Haemophilus influenzae*, *Listeria monocitogenes*, *Neisseria meningitidis*, *Proteus vulgaris*, *Streptococcus agalactiae*, *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Streptococcus pyogenes*, etc.), viruses (*Cytomegalovirus hominis*, *Herpes simplex virus* I and II type, *Human herpes virus* 6, 7 and 8 type, *Parvovirus* B19, *Varicella-Zoster virus,* etc.)

The clinical specificity of **AmpliSens®** ***Pseudomonas aeruginosa*****-screen-titre-FRT** PCR kit was confirmed in laboratory clinical trials.

## 14. REFERENCES


1. Handbook “Sampling, Transportation, and Storage of Clinical Material for PCR diagnostics”, developed by Federal Budget Institute of Science “Central Research Institute for Epidemiology” of Federal Service for Surveillance on Consumers’ Rights Protection and Human Well-Being, Moscow, 2008.
2. Guidelines to the **AmpliSens®** ***Pseudomonas aeruginosa*****-screen-titre-FRT** PCR kit for qualitative detection and quantitation of *Pseudomonas aeruginosa* DNA in the clinical material (blood, blood plasma, oropharyngeal swab, bronchoalveolar lavage, sputum, endotracheal aspirate, urine, prostatic fluid, cerebrospinal fluid, punctate from the lesions of organs and tissues) by the polymerase chain reaction (PCR) with real-time hybridization-fluorescence detection developed by Federal Budget Institute of

Science “Central Research Institute for Epidemiology”.

## 15. QUALITY CONTROL

In compliance with Federal Budget Institute of Science “Central Research Institute for Epidemiology” ISO 13485-Certified Quality Management System, each lot of the **AmpliSens® *Pseudomonas aeruginosa*-screen-titre-FRT** PCR kit has been tested against predetermined specifications to ensure consistent product quality.

## 16. KEY TO SYMBOLS USED

| | | | |
|---|---|---|---|
| REF | Catalogue number | Σ | Sufficient for |
| LOT | Batch code | | Expiration Date |
| RUO | Research use only | | Consult instructions for use |
| VER | Version | | Keep away from sunlight |
| | Temperature limitation | **NCA** | Negative control of amplification |
| | Manufacturer | **C–** | Negative control of extraction |
| | Date of manufacture | **PCE** | Positive control of extraction |
| | Caution | **IC** | Internal control |