

IVD

For *in Vitro* Diagnostic Use

# AmpliSens<sup>®</sup> *Mycoplasma pneumoniae* / *Chlamydomphila pneumoniae*-FRT PCR kit Instruction Manual

AmpliSens<sup>®</sup>

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## 1. INTENDED USE.

AmpliSens<sup>®</sup> *Mycoplasma pneumoniae* / *Chlamydomphila pneumoniae*-FRT PCR kit is an *in vitro* nucleic acid amplification test for qualitative detection of *Mycoplasma pneumoniae* and *Chlamydomphila pneumoniae* DNA in the clinical materials (sputum, nasopharyngeal swabs, throat swabs, bronchi scourage or bronchoalveolar lavage, whole blood, autopsy material) by using real-time hybridization-fluorescence detection.

## 2. PRINCIPLE OF PCR DETECTION.

*Mycoplasma pneumoniae* and *Chlamydomphila pneumoniae* detection by the polymerase chain reaction (PCR) is based on the amplification of pathogen genome specific region using special *Mycoplasma pneumoniae* and *Chlamydomphila pneumoniae* primers. In real-time PCR the amplified product is detected using fluorescent dyes. These dyes are linked to oligonucleotide probes which bind specifically to the amplified product. The real-time PCR monitoring of the fluorescence intensities during the real-time PCR allows the detection of accumulating product without re-opening the reaction tubes after the PCR run. AmpliSens<sup>®</sup> *Mycoplasma pneumoniae* / *Chlamydomphila pneumoniae*-FRT PCR kit is a qualitative test, which uses the principle of endogenous control – amplification of human prothrombin gene fragment. DNA-target selected as endogenous internal control is the fragment of human genome and must be present in a sample in sufficient quantity equivalent to that of cells in the sample (no less than 10<sup>3</sup> genomes). Therefore, endogenous internal control allows not only to monitor stages of the test (DNA extraction and PCR conducting) but also to assess the adequacy of clinical material collection and storage. If the amount of cells in the specimen insufficient, signal of amplification of prothrombin gene will be too low. AmpliSens<sup>®</sup> *Mycoplasma pneumoniae* / *Chlamydomphila pneumoniae*-FRT PCR kit uses “hot-start”, which greatly reduces frequency of nonspecifically primed reactions. “Hot-start” is guaranteed by separation of nucleotides and Taq-polymerase by using wax layer. Wax melting and reaction mix components occur only at 95°C, which greatly diminishes the frequency of nonspecifically primed reactions.

## 3. CONTENT.

AmpliSens<sup>®</sup> *Mycoplasma pneumoniae* / *Chlamydomphila pneumoniae*-FRT PCR kit is produced in 2 forms:

AmpliSens<sup>®</sup> *Mycoplasma pneumoniae* / *Chlamydomphila pneumoniae*-FRT PCR kit variant FRT (for use with RG) REF R-B42-4x(RG).

AmpliSens<sup>®</sup> *Mycoplasma pneumoniae* / *Chlamydomphila pneumoniae*-FRT PCR kit variant FRT (for use with iQ) REF R-B42-4x(iQ).

AmpliSens<sup>®</sup> *Mycoplasma pneumoniae* / *Chlamydomphila pneumoniae*-FRT PCR kit, variant FRT includes:

Reagent	Description	Volume (ml)	Quantity
PCR-mix-1-FEP/FRT <i>Mycoplasma pneumoniae</i> / <i>Chlamydomphila pneumoniae</i> ready-to-use single-dose test tubes (under wax)	colorless, clear liquid	0.008	55 tubes of 0.2 ml
PCR-mix-2-FL	colorless, clear liquid	0.77	1 tube
Positive Control DNA <i>Mycoplasma pneumoniae</i> (C <sub>M,P</sub> +)	colorless, clear liquid	0.1	1 tube
Positive Control DNA <i>Chlamydomphila pneumoniae</i> (C <sub>C,P</sub> +)	colorless, clear liquid	0.1	1 tube
Positive Control DNA human	colorless, clear liquid	0.2	1 tube
DNA-buffer	colorless, clear liquid	0.5	1 tube
Negative Control (C-)*	colorless, clear liquid	1.2	1 tube

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

AmpliSens<sup>®</sup> *Mycoplasma pneumoniae* / *Chlamydomphila pneumoniae*-FRT PCR kit is sufficient for 55 reactions, including controls.

## 4. ADDITIONAL REQUIREMENTS.

- DNA isolation kit
- Disposable powder-free gloves
- Pipettes (adjustable)
- Sterile pipette tips with aerosol filters ( up to 200 µl)
- Tube racks
- Vortex mixer
- Desktop centrifuge with rotor for 2 ml reaction tubes
- PCR box
- Rotor-Gene™ 3000 or Rotor-Gene™ 6000 (Corbett Research, Australia) Instrument; iQ5 or iQCycler (BioRad, USA) Instrument

- Disposable polypropylene microtubes for PCR with 0.5 ml (0.2) capacity.
- Refrigerator for 2–8 °C
- Deep-freezer with temperature not more than –16°C.
- Waste bin for used tips.

## 5. GENERAL PRECAUTIONS.

The user should always pay attention to the following:

- Use sterile pipette tips with aerosol filters 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 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 mucose membranes. If any of these solutions 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.
- Workflow in the laboratory must proceed in a one directional manner, beginning in the Extraction Area and moving to the Amplification and Detection Area. 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.

## 6. SAMPLING AND HANDLING.



Obtaining samples of biological materials for PCR-analysis, transportation and storage is described in manufacturer's handbook [2]. It is recommended that this handbook is read before starting work.

**AmpliSens<sup>®</sup> *Mycoplasma pneumoniae* / *Chlamydomphila pneumoniae*-FRT** PCR kit is intended for the analysis of DNA extracted with DNA isolation kits from sputum, nasopharyngeal swabs, throat swabs, bronchi scourage or bronchoalveolar lavage, whole blood, autopsy material.

## 7. PROTOCOL.

### 7.1. DNA Isolation

It's recommended to use the following nucleic acid extraction kits:

- "DNA-sorb-B", **REF** K1-2-100 (for secret of the prostate gland).



Carry the DNA isolation according to the manufacturer's instructions.

### 7.2. Preparing the PCR.

Total reaction volume is 25 µl, the volume of DNA sample is 10 µl.

#### 7.2.1 Preparing tubes for PCR.

##### Variant FRT

1. Prepare the required number of the tubes with **PCR-mix-1-FEP/FRT *Mycoplasma pneumoniae* / *Chlamydomphila pneumoniae*** and wax for amplification of DNA from clinical and control samples.
2. Add 7 µl of **PCR-mix-2-FL** to the surface of the wax layer of each tube, that it does not fall under the wax and mix with **PCR-mix-1-FEP/FRT *Mycoplasma pneumoniae* / *Chlamydomphila pneumoniae***.
3. Using tips with aerosol filter add 10 µl of **DNA samples** obtained from clinical or control samples at the DNA extraction stage into prepared tubes.



The tubes with **PCR-mix-1-FEP/FRT *Mycoplasma pneumoniae* / *Chlamydomphila pneumoniae*** that are not used at the moment should be stored away from light.

4. Carry the control amplification reactions:

**NCA** Add 10 µl of **DNA-buffer** to the tube labeled NCA (Negative Control of Amplification).

**C<sup>+</sup><sub>M. p.</sub>** Add 10 µl of **Positive Control DNA *Mycoplasma pneumoniae*** to the tube labeled C<sup>+</sup><sub>M. p.</sub> (Positive Control of Amplification).

**C<sup>+</sup><sub>C. p.</sub>** Add 10 µl of **Positive Control DNA *Chlamydomphila pneumoniae*** to the tube labeled C<sup>+</sup><sub>C. p.</sub> (Positive Control of Amplification).

**C<sup>+</sup><sub>DNA human</sub>** -Add 10 µl of **Positive Control DNA human** to the tube labeled C<sup>+</sup><sub>DNA human</sub> (Positive Control of Amplification).

### 7.2.2. Amplification

#### 7.2.2.1. RG

1. Program the Rotor-Gene™ according to manufacturer's manual and Appendix 1.

2. Create a temperature profile on your Rotor-Gene™ instrument as follows:

- |    |           |                            |
|----|-----------|----------------------------|
| a. | Hold      | 95 °C – 5 min              |
| b. | Cycling   | 95 °C – 10 sec             |
|    |           | 63 °C – 30 sec             |
|    |           | 72 °C – 10 sec             |
|    |           | Cycles repeats – 10 times  |
| c. | Cycling 2 | 95 °C – 10 sec             |
|    |           | 60 °C – 30 sec - Detection |
|    |           | 72 °C – 10 sec             |
|    |           | Cycles repeats – 35 times  |

3. Fluorescence detection is on the 2-nd pass (**60°C**) in FAM/Green, JOE/Yellow and ROX/Orange fluorometer channels.

4. Make the adjustment of the fluorescence channel sensitivity according to Appendix 1.

#### 7.2.2.2. iQ

1. Program the iQ™ according to manufacturer's manual and Appendix 2.

2. Create a temperature profile on your iQ™ instrument as follows:

- |    |           |                            |
|----|-----------|----------------------------|
| a. | Hold      | 95 °C – 5 min              |
| b. | Cycling   | 95 °C – 15 sec             |
|    |           | 63 °C – 45 sec             |
|    |           | 72 °C – 15 sec             |
|    |           | Cycles repeats – 10 times  |
| c. | Cycling 2 | 95 °C – 15 sec             |
|    |           | 60 °C – 45 sec - Detection |
|    |           | 72 °C – 15 sec             |
|    |           | Cycles repeats – 35 times  |

3. Fluorescence detection is on the 2-nd pass (**60°C**) in FAM, JOE and ROX fluorometer channels.

4. Make the adjustment of the fluorescence channel sensitivity according to Appendix 2.

## 8. DATA ANALYSIS.

**RG.** *Mycoplasma pneumoniae* DNA is detected on the FAM /Green fluorescence channel, *Chlamydomphila pneumoniae* DNA – on the ROX/Orange channel, DNA human - on the JOE/Yellow channel.

See **Appendix 1** for data analysis settings for Rotor-Gene™ 3000 or Rotor-Gene™ 6000.

**iQ.** *Mycoplasma pneumoniae* DNA is detected on the FAM fluorescence channel, *Chlamydomphila pneumoniae* DNA – on the ROX channel, DNA human (internal control) - on the JOE channel.

See **Appendix 2** for data analysis settings for iQ5 or iQICycler.

### 8.2. Results interpretation

The results are interpreted by the device software by the crossing (or not) of the fluorescence curve with the threshold line.

Results are accepted as relevant if both positive and negative controls of amplification along with negative control of extraction are passed.

### Results for controls

Control	Stage for control	Ct value on channel			Interpretation
		FAM/Green / result	ROX/Orange / result	JOE/Yellow / result	
C-	DNA isolation	Neg	Neg	Neg	OK
NCA	Amplification	Neg	Neg	Neg	OK
C+ <sub>M, p.</sub>	Amplification	Pos (< X)	Neg	Neg	OK
C+ <sub>C, p.</sub>	Amplification	Neg	Pos (< Y)	Neg	OK
C+ <sub>DNA human</sub>	Amplification	Neg	Neg	Pos (< Z)	OK

\*For X, Y, Z values see Appendix 1, 2

- The sample is considered positive if Ct values are defined in the results grid or FAM/Green and ROX/Orange channels.
- The sample is considered negative if Ct value is not defined in the results grid (the fluorescence curve does not cross the threshold line) in FAM/Green or ROX/Orange channels and in the results grid in the JOE/Yellow channel the Ct value doesn't exceed Z.

### 9. TROUBLESHOOTING.

Results of analysis are not being registered in the following cases:

- If no signal is detected for Positive Controls of amplification, it can suggest incorrect programming of the temperature profile, incorrect configuration of the PCR reaction or storage conditions for kit components has not complied with manufacturer instruction, or the reagents kit has expired. Programming of devices (see 7.2.2.1. and 7.2.2.2.), storage conditions, and the expiration date of the reagents should be checked, and then the PCR should be repeated.
- If the signal is registered in Negative Control of extraction (C-) on FAM/Green channel and/or in Negative Control of amplification (NCA) in any of the channels, it indicates the contamination of reagents or samples. In this case results of the analysis for all samples are considered invalid. It is necessary to repeat the analysis of all tests, and also to take measures to detect and eliminate the source of contamination.
- If Ct value for DNA human in results grid (channel JOE/Yellow) is more than Z, the sample extraction process should be repeated. If the same result is achieved, it can suggest incorrect sampling or storage of clinical material. Sampling should be repeated.

If you have any further questions or if you encounter problems, please contact our Authorized representative in the European Community.

### 10. STABILITY AND STORAGE.

All components of the **AmpliSens<sup>®</sup> Mycoplasma pneumoniae / Chlamydomphila pneumoniae-FRT** PCR kit are to be stored away from the light and at the temperature between 2 and 8°C, when not in use. They also must be stable until the expiry date stated on the label.

### 11. SPECIFICATIONS.

#### 11.1. Sensitivity.

Analytical Sensitivity of **AmpliSens<sup>®</sup> Mycoplasma pneumoniae / Chlamydomphila pneumoniae-FRT** PCR kit is no less than 1x10<sup>3</sup> genome equivalents per 1 ml of sample (GE/ml).



The claimed analytical features of **AmpliSens<sup>®</sup> Mycoplasma pneumoniae / Chlamydomphila pneumoniae-FRT** PCR kit are guaranteed only when additional reagents kits "DNA-sorb-B" (manufactured by Federal State Institution of Science Central Research Institute of Epidemiology) are used.

#### 11.2. Specificity.

Specificity of **AmpliSens<sup>®</sup> Mycoplasma pneumoniae / Chlamydomphila pneumoniae-FRT** PCR kit is assured by selection of specific primers and probes, as well as the selection of strict reaction conditions. The primers and probes were checked for possible homologies to all in gene banks published sequences by sequence comparison analysis. Specificity of **AmpliSens<sup>®</sup> Mycoplasma pneumoniae / Chlamydomphila pneumoniae-FRT** PCR kit was confirmed in laboratory clinical trials.

### 12. REFERENCES.

- Handbook "Sampling, transportation, storage of clinical material for PCR diagnostics", developed by Federal State Institution of Science Central Research Institute of Epidemiology of Federal Service for Surveillance on Consumers' Rights Protection and Human Well-Being, Moscow, 2008.

### 13. QUALITY CONTROL.

In compliance with Federal State Institution of Science "Central Research Institute of Epidemiology" ISO 13485 –certified Quality Management System, each lot of **AmpliSens<sup>®</sup> Mycoplasma pneumoniae / Chlamydomphila pneumoniae-FRT** PCR kit has been tested against predetermined specifications to ensure consistent product quality.

### 14. EXPLANATION OF SYMBOLS.



Manufacturer



Use by



For in Vitro Diagnostic Use



Catalogue number



Contains sufficient for <n> tests



Consult instructions for use



For working with Rotor-Gene™ 3000/6000



Positive control



Temperature limitation



Batch code



Version



Internal Control complex



Authorized representative in the European Community.



Caution, consult accompanying documents



For working with iQ5, iQ.iCycler



Negative control