

IVD

For *in Vitro* Diagnostic Use

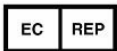
AmpliSens® *Mycoplasma pneumoniae* / *Chlamydomphila pneumoniae*-FEP PCR kit

Instruction Manual

AmpliSens®

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1. INTENDED USE.

AmpliSens® *Mycoplasma pneumoniae* / *Chlamydomphila pneumoniae*-FEP 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 end-point hybridization-fluorescence detection of amplified products.

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 end point PCR, the amplified product is detected using fluorescent dyes. These dyes are linked to oligonucleotide probes which bind specifically to the amplified product in ordinary thermocycler. A multi channel rotor-type fluorometer is specially designed to detect fluorescent excitation from the fluorophores in a reaction mix after PCR. It allows the accumulating product detection without re-opening the reaction tubes after the PCR run. **AmpliSens® *Mycoplasma pneumoniae* / *Chlamydomphila pneumoniae*-FEP** 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. 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. The wax melting and reaction mix component occurs only at 95°C.

3. CONTENT.

AmpliSens® *Mycoplasma pneumoniae* / *Chlamydomphila pneumoniae*-FEP PCR kit is produced in 2 forms:

AmpliSens® *Mycoplasma pneumoniae* / *Chlamydomphila pneumoniae*-FEP PCR kit (tubes 0.5 ml), **REF** B42-50-R0,5-FEP-CE.

AmpliSens® *Mycoplasma pneumoniae* / *Chlamydomphila pneumoniae*-FEP PCR kit (tubes 0.2 ml), **REF** B42-50-R0,2- FEP-CE.

AmpliSens® *Mycoplasma pneumoniae* / *Chlamydomphila pneumoniae*-FEP PCR kit includes:

Reagent	Description	Volume (ml)	Amount
PCR-mix-1-FEP/FRT <i>Mycoplasma pneumoniae</i> / <i>Chlamydomphila pneumoniae</i> ready-to-use single-dose test-tubes (under wax)	colorless, clear fluid	0.008	55 tubes of 0.2 or 0.5 ml
PCR-mix-2-FL	colorless, clear fluid	0.77	1 tube
PCR-mix-Background	colorless, clear fluid	0.5	1 tube
Mineral oil for PCR	colorless, viscous fluid	4.0	1 vial
Positive Control DNA <i>Mycoplasma pneumoniae</i> (C_{M,p}+) 	colorless, clear fluid	0.1	1 tube
Positive Control DNA <i>Chlamydomphila pneumoniae</i> (C_{C,p}+) 	colorless, clear fluid	0.1	1 tube
Positive Control DNA human	colorless, clear fluid	0.2	1 tube
DNA-buffer	colorless, clear fluid	0.5	1 tube
Negative Control (C-)*	colorless, clear fluid	1.2	1 tube

* must be used in the isolation procedure as Negative Control of Extraction (see “DNA-sorb-B”, [REF](#) K1-2-50-CE protocols).

AmpliSens® *Mycoplasma pneumoniae* / *Chlamydomphila pneumoniae*-FEP PCR kit is intended for 55 reactions, including controls.

4. ADDITIONAL REQUIREMENTS.

- DNA isolation kit.
- Disposable powder-free gloves and laboratory coat.
- Pipettes (adjustable).
- Sterile pipette tips with aerosol barriers (up to 200 µl).
- Tube racks.
- Vortex mixer.
- Desktop centrifuge with rotor for 2 ml reaction tubes.
- PCR box.
- Personal thermocyclers (for example, Gradient Palm Cycler (Corbett Research, Australia), Maxygene (Axygen, USA) or equivalent instrument).
- Fluorometer ALA-1/4 (“Biosan”, Latvia) or equivalent 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 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, protect eyes while samples and reagents handling. 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 samples and unused reagents in compliance with local authorities requirements.
- Samples should be considered potentially infectious and handled in a biological cabinet in compliance 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 contact with the skin, eyes and mucose membranes. If skin, eyes and mucose membranes contact immediately flush with water, seek medical attention
- 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 move 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 [1]. It is recommended that this handbook is read before starting work.

AmpliSens® *Mycoplasma pneumoniae* / *Chlamydomphila pneumoniae*-FEP PCR kit is intended for analysis of DNA

extracted by using 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](#) K1-2-50-CE.



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

7.2. Preparing the PCR.

Total reaction volume - **25 µl**, volume of DNA sample - **10 µl**.

7.2.1 Preparing tubes for PCR.

1. Prepare the required number of 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 ensuring that it does not fall under the wax and mix with **PCR-mix-1-FEP/FRT *Mycoplasma pneumoniae* / *Chlamydomphila pneumoniae***.
3. Add above **1** drop of **mineral oil for PCR** (about **25 µl**).
4. Prepare 2 tubes with **PCR-mix-1-FEP/FRT *Mycoplasma pneumoniae* / *Chlamydomphila pneumoniae*** and mark them as **Background**. Add **17 µl** of **PCR-mix-Background** to the surface of wax layer of each tube, ensuring that it does not fall under the wax and mix with **PCR-mix-1-FEP/FRT *Mycoplasma pneumoniae* / *Chlamydomphila pneumoniae***. Add above **1** drop of **mineral oil for PCR**.
5. Using tips with aerosol barrier, add **10 µl** of **DNA samples** obtained from clinical or control samples at the DNA extraction stage.
6. Carry the control amplification reactions:

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

C⁺_{M. pneumoniae} Add **10 µl** of **Positive Control DNA *Mycoplasma pneumoniae*** to the tube labeled C⁺_{M. p.} (Positive Control of Amplification).

C⁺_{C. pneumoniae} Add **10 µl** of **Positive Control DNA *Chlamydomphila pneumoniae*** to the tube labeled C⁺_{c. p.} (Positive Control of Amplification).

C⁺_{DNA human} Add **10 µl** of **Positive Control DNA human** to the tube labeled C⁺_{DNA human} (Positive Control of Amplification).

7.2.2 Amplification.

Run the following program on the thermocycler (see Table 1). When the temperature reaches 95 °C (pause regimen), insert tubes to cells of amplifier and press the button to continue.

It is recommended to sediment drops from walls of tubes by short vortex (1–3 sec) before placing them in the thermocycler.

Table 1

Programming thermocyclers at DNA amplification of *Mycoplasma pneumoniae* and *Chlamydomphila pneumoniae*

Step	Thermocyclers with active temperature adjustment:						Thermocyclers with block temperature adjustment: "Uno-2" ("Biometra")		
	"Terzik" (DNA-Technology)			"GeneAmp PCR System 2700" ("ABI"), "Gradient Palm Cyclor" ("Corbett Research"), "MyCycler" ("BioRad"), "Maxygene" ("Axygen")					
	Temperature	Time	Cycles	Temperature	Time	Cycles	Temperature	Time	Cycles
0	95 °C	pause		95 °C	pause		95 °C	pause	
1	95 °C	5 min	1	95 °C	5 min	1	95 °C	5 min	1
2	95 °C	10 sec	42	95 °C	10 sec	42	95 °C	25 sec	42
	63 °C	20 sec		63 °C	25 sec		63 °C	40 sec	
	72 °C	20 sec		72 °C	25 sec		72 °C	25 sec	
3	72 °C	1 min	1	72 °C	1 min	1	72 °C	1 min	1
4	4 °C	storage		4 °C	storage		10 °C	storage	

8. DATA ANALYSIS.

Detection is conducted on ALA-1/4 fluorescence detector.



Please read Aladin Operating Manual before use of this kit.

Program the detector according to the manufacturer's manual and Appendix 1.

8.1. Results interpretation.

1. When the analysis is complete the results are automatically shown in the table as follows:

pos – positive result;

neg – negative result;

eq – equivocal result (signal at the channel for detection of specific cDNA exceed threshold value for negative samples, but does not exceed threshold value for positive samples (signal is in grey zone);

nd – invalid result (specific signal and IC signal does not detect (does not exceed threshold value) in the sample).

Result of the analysis is considered reliable only if both Positive and Negative Controls of amplification as well as Negative Control of extraction are passed (Table 2).

Table 2

Results for controls

Control	Stage for control	Result of automatic interpretation			Interpretation
		FAM channel (Mycoplasma pneumoniae)	FAM channel (Chlamidophila pneumoniae)	HEX channel (IC)	
C-	DNA isolation	«Myc. pn. – nd»	«Chl. pn. – nd»	-	OK
NCA	Amplification	«Myc. pn. – nd»	«Chl. pn. – nd»	-	OK
C ⁺ <i>M. pneumoniae</i>	Amplification	«Myc. pn. – pos»	«Chl. pn. – neg»	-	OK
C ⁺ <i>C. pneumoniae</i>	Amplification	«Myc. pn.- neg»	«Chl. pn. – pos»	-	OK
C ⁺ DNA human	Amplification	«Myc. pn.- neg»	«Chl. pn. – neg»	+	OK

9. TROUBLESHOOTING.

If analysis results are not obtained as per the following examples:

1. Preparing the PCR and detection should be repeated for samples with result **nd** (excepting NCA). In the case of analogous result, it is necessary to repeat the sample analysis, beginning with extraction stage. For the NCA sample the result **nd** is normal.
2. Preparing the PCR and detection should be repeated for samples with result **eq**. In the case of analogous result, the samples are considered to be positive.
3. No positive signal in positive control of PCR (PC) can indicate incorrect programming of the temperature profile of the thermocycler, incorrect configuration of the PCR reaction, or storage conditions for kit components not complying with manufacturer instruction, or reagent kit has expired. Check programming of the thermocycler (see 7.2.2.), storage conditions, and the expiration date of the reagents and repeat PCR reaction once again for all samples.
4. Positive signal in negative controls (C-, NCA) indicates the reagent or sample contamination. In this case results of the analysis 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 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[®] Mycoplasma pneumoniae / Chlamydomphila pneumoniae-FEP** 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[®] Mycoplasma pneumoniae / Chlamydomphila pneumoniae-FEP** PCR kit is no less than 1x10³ genome equivalents per 1 ml of sample (GE/ml).



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

11.2. Specificity.

Specificity of **AmpliSens[®] Mycoplasma pneumoniae / Chlamydomphila pneumoniae-FEP** PCR kit is ensured by selection of specific primers and probes, as well as the selection of strict reaction conditions. The primers and probes have been checked for possible homologies to all in gene banks published sequences by sequence comparison analysis. Specificity of **AmpliSens[®] Mycoplasma pneumoniae / Chlamydomphila pneumoniae-FEP** PCR kit was confirmed in laboratory clinical trials.

















12. REFERENCES.

1. Handbook "Sampling, transportation, storage of clinical material for PCR diagnostics", developed by Federal

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® *Mycoplasma pneumoniae* / *Chlamydophila pneumoniae*-FEP** PCR kit has been tested against predetermined specifications to ensure consistent product quality.

14. EXPLANATION OF SYMBOLS.

	Manufacturer		Temperature limitation
	Use by		Batch code
	For <i>in Vitro</i> Diagnostic Use		Version
	Catalogue number		Internal Control complex
	Contains sufficient for <n> tests		Authorized representative in the European Community.
	Consult instructions for use		Caution, consult accompanying documents
	For working with Rotor-Gene™ 3000/6000		For working with iQ5, iQ iCycler
	Positive control		Negative control