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For Professional Use Only

# MC-EPh

# PCR kit

## Instruction Manual

**AmpliSens®**

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## 1. INTENDED USE

**MC-EPh PCR kit** is an *in vitro* nucleic acid amplification test for qualitative detection of *Mycoplasma* microorganisms DNA in the biological material by using electrophoretic detection of the amplified products in agarose gel.

This kit is used only for scientific research.

## 2. PRINCIPLE OF PCR DETECTION

*Mycoplasma* detection by the polymerase chain reaction (PCR) is based on the amplification of pathogen DNA specific region using special *Mycoplasma* primers. After PCR the amplified product is detected in agarose gel. **MC-EPh** PCR kit uses “hot-start”, which greatly reduces the frequency of nonspecifically primed reactions. “Hot-start” is guaranteed by separation of nucleotides and Taq-polymerase by using a wax layer. Wax melts and reaction components mix only at 95 °C.

## 3. CONTENT

**MC-EPh** PCR kit is produced in 2 forms:

MC-EPh PCR kit variant 50 R (tubes 0.2 ml) **REF** B72-50-R0,2-CE;

MC-EPh PCR kit variant 50 R (tubes 0.5 ml) **REF** B72-50-R0,5-CE.

**MC-EPh** PCR kit variant 50 R includes:

| ***Reagent*** | ***Description*** | ***Volume (ml)*** | ***Quantity*** |
|---|---|---|---|
| **PCR-mix-1-R MYC-COM** ready-to-use single-dose test tubes (*under wax)* | colorless clear liquid | 0.005 | 55 tubes of 0.5 or 0.2 ml |
| **PCR-mix-2 blue** | blue clear liquid | 0.6 | 1 tube |
| **Mineral oil for PCR** | colorless viscous liquid | 2.0 | 1 dropper bottle |
| **Positive Control DNA *Mycoplasma hominis* (C+$_{M.hominis}$)** | 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 extraction procedure as Negative Control of Extraction.

**MC-EPh** PCR kit variant 50 R is intended for 55 reactions, including controls.

## 4. ADDITIONAL REQUIREMENTS

- DNA extraction kit.

- Agarose gel detection kit.
- Disposable powder-free gloves and laboratory coat.
- Pipettes (adjustable).
- Sterile pipette tips with aerosol barriers (up to 200 µl).
- PCR box.
- Tube racks.
- Personal thermocyclers (for example, Terzik (DNA-Technology, Russia).
- Refrigerator with temperature 2-8 °C.
- Deep-freezer with temperature not more than minus 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 and handle amplicons away from all other reagents.
- Thaw all components thoroughly at room temperature before starting detection.
- When thawed, mix the components and centrifuge briefly.
- Use disposable gloves, laboratory coats, 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 compliance with local authorities requirements.
- Samples 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 contact with the skin, eyes and mucosa. If skin, eyes and mucosa 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.

- Observe the rules and instructions to prevent semination of the environment objects.

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

## 6. SAMPLING AND HANDLING

**MC-EPh** PCR kit is intended for analysis of DNA extracted with DNA extraction kits from the biological material:

- Nasal swabs, conjunctive swabs, outflows.
- Joint synovial fluid.
- Dotter substance, embryo allantoic fluid.
- Parenchymatous organs, trachea, air sacs.
- Whole blood.
- Medicinal serum, cell cultures ($1x10^6$ cells for 1 ml).

### 6.1. Material sampling

Blood with 6 % EDTA (20:1).

The parts of tissues and organs – about 1x1x1 (cm) or less.

Materials are to be analyzed the next day after sampling if they are stored at the temperature 2-8 °C.

Materials can be stored at the temperature not more than minus 16 °C for one month.

### 6.2. Preparation of samples

Whole blood samples with EDTA, medicinal serum and cell cultures are used for DNA extraction without treatment.

Other fluid samples (1.5-ml). Centrifuge at 10000 rpm for 5 min. Carefully remove and discard the supernatant using a tip with aerosol barrier leaving about 200 µl of the liquid on the sediment. If the pellet is too small, add more material (1.5 ml) and repeat centrifugation. Suspend the pellet in supernatant and use 100 µl for DNA extraction.

Parenchymatous organs, trachea, air sacs should be ground in a sterile porcelain mortar or glass homogenizer. Add equal volume of saline and thoroughly homogenize. Incubate at room temperature for 30 min. Transfer 100 µl of the upper part of prepared suspension to a sterile tube for DNA extraction.

## 7. WORKING CONDITIONS

**MC-EPh** 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:

- ”DNA-sorb-B”, **REF** K1-2-50-CE

Carry out the amplification according to the manufacturer instruction. The volume of clinical sample is 100 µl.

Add 100 µl of Negative Control (C-) into tube used as Negative Control of Extraction.

Add 90 µl of Negative Control (C-) and 10 µl of Positive Control DNA *Mycoplasma hominis* (C+) into tube used as Positive Control of Extraction.

### 8.2. Preparing the PCR

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

#### 8.2.1. Detection of *Mycoplasma* DNA

1. Prepare the required number of PCR tubes with **PCR-mix-1-R MYC-COM** and wax for amplification of DNA from clinical and control samples.
2. Add **10 µl** of **PCR-mix-2 blue** 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-R MC**.
3. Add above **1 drop** of **mineral oil for PCR** (about **25 µl**).
4. Using tips with aerosol barrier add **10 µl** of **DNA samples** obtained from clinical or control samples.
5. Carry out the control amplification reactions:

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

C+ -Add **10 µl** of **Positive Control DNA *Mycoplasma hominis*** to the tube labeled C+.

#### 8.2.2. Amplification of *Mycoplasma* DNA

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

It is recommended to precipitate drops from walls of tubes by short vortexing (1–3 sec) before their insertion in a thermocycler.

Table 1

**Programming thermocyclers for *Mycoplasma* DNA amplification**

| | Thermocyclers with active temperature adjustment | | | | | | Thermocyclers with block temperature adjustment | | |
|---|---|---|---|---|---|---|---|---|---|
| | **Terzik (DNA-Technology), Omn-E (Hybaid)** | | | **GeneAmp PCR System 2700 (Applied Biosystems), Palm Cycler (Corbett Research)** | | | **Ampli-3 (Biocom), Biometra, MiniCycler, PTC-100 (MJ Research)** | | |
| Step | Temperature, °C | Time | Cycles | Tempera-ture, °C | Time | Cycles | Tempera-ture, °C | Time | Cycles |
| 0 | **95** | pause | | **95** | pause | | **95** | pause | |
| 1 | **95** | 5 min | 1 | **95** | 5 min | 1 | **95** | 5 min | 1 |
| 2 | **95** | 10 s | 41 | **95** | 10 s | 41 | **95** | 1 min | 41 |
| | **61** | 10 s | | **61** | 25 s | | **61** | 1 min | |
| | **72** | 10 s | | **72** | 25 s | | **72** | 1 min | |
| 3 | **72** | 1 min | 1 | **72** | 1 min | 1 | **72** | 1 min | 1 |
| 4 | **10** | storage | | **4** | storage | | **10** | storage | |

After the reaction is finished PCR tubes must be collected and sent to the room for PCR products analysis.

Analysis of amplification products is performed by separation of DNA fragments in agarose gel.

The amplified samples can be stored for 16 h at room temperature, for 1 week at 2-8 °C (be sure to warm the samples to room temperature before running electrophoresis).

## 9. DATA ANALYSIS

It's recommended to use the following agarose kit for electrophoretic detection:

- "EPh" variant 200, **REF** K5-200-CE.

Analysis of results is based on the presence or absence of specific bands of amplified DNA in agarose gel (1.7 %). The lengths of specific amplified DNA fragments are:

- *Mycoplasma* – 509 bp (base pairs)

Put the protective mask or use the glass barrier while watching and photographing the gel.

Start analysis from results for controls (see Table 2).

Table 2

**Results for controls**

| Control | Controlled step | Specific bands in the agarose gel 509 bp | Interpretation |
|---|---|---|---|
| C– | DNA extraction | No | OK |
| PCE | DNA extraction | **Yes** | OK |
| NCA | Amplification | No | OK |
| C+ | Amplification | **Yes** | OK |

- The sample is considered to be positive if the band of 509 bp is present in agarose gel.
- The sample is considered to be negative if the band of 509 bp is absent.
- Besides specific bands, the fuzzy bands of primer dimers can be seen in lanes, they are situated lower than the level of 100 bp.

## 10. TROUBLESHOOTING

Analysis results are not taken into account in the following cases:

1. If the results of control samples do not correspond to the listed above (Table 2), then the tests should be repeated.
2. If the specific 509-bp band is absent in lanes corresponding to positive control (PCE, C+), it may be mistake of preparation of reagents, amplification or program error of thermocycler.
3. If nonspecific bands appear in lanes at different levels, it may be caused by the lack of “hot start” or a false temperature regime in thermocycler.
4. If the specific 509-bp band appears in lanes corresponding to negative control (NCA, C–), it means that reagents or samples are contaminated. In such cases, analysis results must be considered as irrelevant. Test analysis should be repeated and measures for detecting contamination source must be taken.

## 11. TRANSPORTATION

**MC-EPh** PCR kit should be transported at 2–8 ºC for no longer than 5 days. Once received, the PCR kit should be dekitted according to the indicated storage conditions.

## 12. STABILITY AND STORAGE

All components of **MC-EPh** PCR kit are to be stored are to be stored at 2–8 °C when not in use.. All components of the **MC-EPh** PCR kit are stable until labeled expiration date. The shelf life of reagents before and after the first use is the same, unless otherwise stated.

## 13. SPECIFICATIONS

### 13.1. Sensitivity

Analytical Sensitivity of **MC-EPh** PCR kit is no less than $1x10^3$ copies per 1 ml of a sample (cop/ml).

The claimed analytical features of MC-EPh PCR kit are guaranteed only when additional kits of reagents, “DNA-sorb-B” and “EPh” (manufactured by Federal State Budget of Science Central Research Institute for Epidemiology) are used.

### 13.2. Specificity

Specificity of **MC-EPh** PCR kit is ensured by selection of specific primers and strict reaction conditions as well as laboratory and clinical trials.

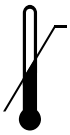
## 14. REFERENCES

1. García, M., M. W. Jackwood, S. Levisohn, and S. H. Kleven. Detection of *Mycoplasma gallisepticum*, *M. synoviae*, and *M. iowae* by multi-species polymerase chain reaction and restriction fragment length polymorphism. Avian Dis 39: 606–616.1995.

## 15. QUALITY CONTROL

In compliance with Federal Budget Institution of Science Central Research Institute for Epidemiology ISO 13485 – certified Total Quality Management System, each lot of **MC-EPh** PCR kit is tested against predetermined specifications to ensure consistent product quality.

## 16. KEY TO SYMBOLS USED

| | | | |
|---|---|---|---|
| REF | Catalogue number |  | Caution |
| LOT | Batch code |  | Sufficient for |
| RUO | Research use only |  | Expiration Date |
| VER | Version |  | Consult instructions for use |
|  | Temperature limitation |  | Keep away from sunlight |
|  | Upper limit of temperature | **NCA** | Negative control of amplification |
|  | Manufacturer | **C–** | Negative control of extraction |
|  | Date of manufacture | **C+** | Positive control of amplification |
| **FBIS CRIE** | Federal Budget Institute of Science “Central Research Institute for Epidemiology” | **IC** | Internal control |

## List of Changes Made in the Instruction Manual

| VER | Location of changes | Essence of changes |
|---|---|---|
| 25.01.12 LA | Cover page | The phrase “For Professional Use Only” was added |
| | Content | New sections “Working Conditions” and “Transportation” were added |
| | | The “Explanation of Symbols” section was renamed to “Key to Symbols Used” |
| | Stability and Storage | The information about the shelf life of reagents before and after the first use was added |
| | Key to Symbols Used | The explanation of symbols was corrected |
| | Cover page, text | The name of Institution was changed to Federal Budget Institute of Science “Central Research Institute for Epidemiology” |
| 11.02.14 ChA | Front page, Key to symbols used | Symbol IVD was changed to RUO |
| 13.10.14 PM | Content | “Positive Control DNA *Mycoplasma hominis* (C+)” was changed to “Positive Control DNA *Mycoplasma hominis* ($C+_{M.hominis}$)” |