CE

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For Professional Use Only

AmpliSens® *MBT*-EPh

PCR kit

Instruction Manual

AmpliSens®

EC REP

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TABLE OF CONTENTS

1. INTENDED USE

AmpliSens® *MBT*-EPh PCR kit is an *in vitro* nucleic acid amplification test for qualitative detection of *Mycobacterium tuberculosis complex (Mycobacterium tuberculosis, Mycobacterium bovis, Mycobacterium bovis BCG, Mycobacterium africanum, Mycobacterium microti)* DNA in the clinical material by using electrophoretic detection of the amplified products in agarose gel.

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

2. PRINCIPLE OF PCR DETECTION

Mycobacterium tuberculosis complex detection by the polymerase chain reaction (PCR) is based on the amplification of pathogen DNA specific region using special *Mycobacterium tuberculosis complex* primers. After PCR the amplified product is detected in agarose gel. **AmpliSens® *MBT*-EPh** PCR kit is a qualitative test, which contain the Internal Control (IC). It must be used in the extraction procedure in order to control the extraction process of each individual sample and to identify possible reaction inhibition. **AmpliSens® *MBT*-EPh** 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 chemically modified polymerase (TaqF). Chemically modified polymerase (TaqF) is activated by heating at 95 ºC for 15 min.

3. CONTENT

AmpliSens® *MBT*-EPh PCR kit is produced in 2 forms:

AmpliSens® *MBT*-EPh PCR kit variant 100 R (tubes of 0.5 ml volume), **REF** B15-100-R0,5-CE.

AmpliSens® MBT-EPh PCR kit variant 100 R (tubes of 0.2 ml volume), **REF** B15-100-R0,2-CE.

AmpliSens® *MBT*-EPh PCR kit variant 100 R includes:

Reagent	***Description***	***variant 100 R***	
		Volume (ml)	***Quantity***
PCR-mix -1-R *Mycobacterium tuberculosis complex*	colorless clear liquid	0.005	110 tubes of 0.5 or 0.2 ml
2.5x PCR-buffer blue	blue clear liquid	1.15	1 tube
Polymerase (TaqF)	colorless clear liquid	0.06	1 tube
Mineral oil for PCR	colorless viscous liquid	4.0	1 dropper bottle
Positive Control DNA *Mycobacterium tuberculosis H37Ra* (C+)	colorless clear liquid	0.2	2 tubes
TE-buffer	colorless clear liquid	0.5	1 tube
Negative Control (C-)*	colorless clear liquid	1.2	2 tubes
Internal Control *Mycobacterium tuberculosis complex* (IC)**	colorless clear liquid	1.0	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 (see DNA-sorb-B, **REF** K1-2-100-CE protocol).

AmpliSens® MBT-EPh PCR kit variant 100 R is intended for 110 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)
- Vortex mixer
- Thermostatic bath or dry block for tubes with controlled temperature and capability to incubate at 25-100 °C
- Tube racks
- PCR box
- Personal thermocyclers (for example, GeneAmp PCR System 2400 (Applied Biosystems), GeneAmp PCR System 2700 (Applied Biosystems), UNO II (Biometra), MiniCycler (BioRad), PTC-100 (MJ Research), MaxyGene (Axygen) or equivalent)
- Disposable polypropylene microtubes for PCR with 0.5 ml (0.2) capacity (for example, Axygen, USA)
- Refrigerator for 2–8 °C.
- Deep-freezer for ≤ –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.

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

6. SAMPLING AND HANDLING

AmpliSens® *MBT*-EPh PCR kit is intended for analysis of DNA extracted by DNA extraction kits from:

- *Bronchoalveolar lavage*
- *Sputum*
- *Urine*

6.1. *Bronchoalveolar lavage* should be placed into disposable tightly screwing polypropylene container (to avoid cell adhesion on container's interior surface) of 5 ml volume or more. Bronchial scourage or bronchoalveolar lavage is to be shaken in source container. Transfer

1 ml of clinic material into marked Eppendorf tube of 1.5 ml volume with using of the tip with aerosol barrier. Spin the tube for 10 min at 10,000 r/min then carefully remove supernatant with using of vacuum aspirator. Keep 100 µl of the sample in the tube.

6.2. *Sputum* should be placed into calibrated screwing disposable container with wide neck (50 ml volume or more). Add Mucolysin to get a dilution 1:5. Shake from time to time. Transfer 1 ml of clinic material into marked Eppendorf tube of 1.5 ml volume with using of the tip with aerosol barrier.

6.3. *Urine* (midstream portion) calibrated screwing disposable container with wide neck (50 ml volume or more). Transfer 5-10 ml of urine into marked screwing tube of 1.5 ml volume with using of the tip with aerosol barrier. Spin the tube for 10 min at 10,000 r/min then carefully remove supernatant with using of vacuum aspirator. Keep 100 µl of the sample in the tube.

Only one freeze-thaw cycle of clinical material is allowed.

7. WORKING CONDITIONS

AmpliSens® *MBT*-EPh PCR kit should be used at 18–25 °C.

8. PROTOCOL

8.1. DNA Extraction

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

- DNA-sorb-B, **REF** K1-2-100-CE.

Please carry out the DNA extraction according to the manufacturer instruction.

Positive Control DNA *Mycobacterium tuberculosis H37Ra* (C+) must be used during DNA extraction procedure. Add 10 µl of PC DNA *Mycobacterium tuberculosis H37Ra* (C+) and 90 µl of Negative Control (C-) in the tube of Positive Control of Extraction.

8.2. Preparing the PCR

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

8.2.1 Preparing tubes for PCR

1. Collect the required quantity of the PCR tubes with **PCR-mix-1-R *Mycobacterium tuberculosis complex*** for amplification of DNA from clinical or control samples.
2. Prepare the reaction mix in 1.5 ml tube as follows (per one reaction):

   10 µl of 2.5x PCR-buffer blue

   0.5 µl of polymerase (TaqF)

   Spin the tube by vortex/centrifuge.
3. Add 10 µl of prepared reaction mix into the PCR tubes.
4. Add above 1 drop of **mineral oil for PCR** (about 15 µl). Close cups and mark the tubes.

8.2.2 Amplification.

1. Use prepared tubes for PCR. Add **10 µl** of **DNA samples**, obtained from clinical or control samples at the stage of DNA extraction, under or directly above the level of oil by tips with aerosol barrier.
2. Carry out the **control amplification reactions:**

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

C+ - Add 10 µl of **Positive Control DNA Mycobacterium tuberculosis H37Ra** diluted 1:10 into the tube for Positive Control of Amplification.

3. Run the following program on the thermocycler (see table 1). When the temperature reaches 95 ºC (pause regimen), insert tubes to cells of amplifier and press button to continue. It is recommended to precipitate drops from walls of tubes by short vortex (1–3 s) before their insertion in thermocycler.

Table 1

Amplification program of *Mycobacterium tuberculosis complex*

	Thermocyclers with active temperature adjustment:			Thermocyclers with block temperature adjustment:		
	GeneAmp PCR System 2400 (Applied Biosystems), Omn-E (Hibaib), MaxyGene (Axygen)			**UNO II (Biometra), MiniCycler (BioRad), PTC-100 (MJ Research), GeneAmp PCR System 2700 (Applied Biosystems)**		
Step	Temperature	Time	Cycles	Temperature	Time	Cycles
0	**95 °C**	pause		**95 °C**	pause	
1	**95 °C**	15 min	1	**95 °C**	15 min	1
2	**95 °C**	20 s	42	**95 °C**	30 s	42
	70 °C	20 s		**70 °C**	40 s	
	72 °C	20 s		**72 °C**	2 min	
3	**72 °C**	2 min	1			1
4	**10 °C**	storage		**10 °C**	storage	

4. Amplification in thermocycler with block temperature adjustment lasts 2 h 30 min, in thermocycler with active temperature adjustment — 1 h 50 min.
5. After the reaction is finished PCR tubes must be collected and sent to the room for PCR products analysis.

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

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

9. DATA ANALYSIS

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

- 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 length of specific amplified DNA fragments is:

- Positive Control DNA of *Mycobacterium tuberculosis* H37Ra - 390 bp

- Internal Control of *Mycobacterium tuberculosis complex* – 750 bp

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

Results interpretation

Table 2

Results for controls

Control	Controlled step	Specific bands in the agarose gel		Interpretation
		390 bp	750 bp	
PCE	DNA extraction	Yes	Yes	OK
C-	DNA extraction	No	Yes	OK
NCA	Amplification	No	No	OK
C+	Amplification	Yes	No	OK

- The sample is considered to be positive for *Mycobacterium tuberculosis complex* DNA if the band of 390 bp is present in agarose gel. The band of IC (750 bp) could be absent in the samples with high concentration of *Mycobacterium tuberculosis complex* DNA.
- The sample is considered to be negative for *Mycobacterium tuberculosis complex* DNA if the band of 390 bp is absent and the band of 750 bp is present.

Besides specific bands the indistinct washed-out bands of primer-dimers may be seen in lanes, they are situated lower than level of 100 bp of nucleotide pairs.

10. TROUBLESHOOTING

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

- If results of control points analysis do not correspond to the listed above (Table 2), then the tests are to be repeated. Remove any reagents that may be suspect.
- If in lanes none of bands of 390 and 750 nucleotide pairs is observed, result of analysis for this sample is irrelevant and investigation of this sample must be repeated from the very beginning. It can be caused by mistake in clinical processing that provoked loss of RNA/DNA or inhibition of RT and/or PCR.
- If in lines nonspecific bands at different levels are presented, it may be caused by lack of “hot start” or false temperature regimen in thermocycler.
- If in lanes corresponding to negative control (NCA, C–) specific band of 390 bp appears it means that reagents or samples contamination has taken place. In such cases results of analysis must be considered as irrelevant. Test analysis must be repeated and measures for detecting contamination source must be undertaken.

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

11. TRANSPORTATION

AmpliSens® *MBT*-EPh PCR kit should be transported at 2–8 ºC for no longer than 5 days.

12. STABILITY AND STORAGE

All components of the AmpliSens® *MBT*-EPh PCR kit (except for polymerase TaqF) are to be stored at 2–8 ºC when not in use. All components of the PCR kit are to be stable until labeled expiration date. The shelf life of reagents before and after the first use is the same, unless otherwise stated.

Polymerase (TaqF) is to be stored at ≤ –16ºC

13. SPECIFICATIONS

13.1. Sensitivity

Analytical Sensitivity of AmpliSens® *MBT*-EPh PCR kit is $1x10^3$ cells/ml. According to clinical trials Analytical Sensitivity of PCR kit is $1x10^3$ genome equivalents of *Mycobacterium tuberculosis complex* per 1 ml in bronchoalveolar lavage or urine and $5x10^3$ genome equivalents of *Mycobacterium tuberculosis complex* per 1ml of sputum.

Claimed analytical features of AmpliSens® *MBT*-EPh PCR kit are guaranteed only when additional kits of reagents DNA-sorb-B and EPh (manufactured by Federal Budget Institution of Science “Central Research Institute for Epidemiology”) are used.

13.2. Specificity

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

14. REFERENCES

1. Manual “Sampling, transportation and storage of clinical material for PCR diagnostics”, developed by Federal Budget Institution of Science “Central Research Institute for Epidemiology”, Moscow, 2008.

15. QUALITY CONTROL

In compliance with Federal Budget Institution of Science “Central Research Institute for Epidemiology” ISO 13485-Certified Quality Management System, each lot of AmpliSens® *MBT*-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
IVD	*In vitro* diagnostic medical device		Expiration Date
VER	Version		Consult instructions for use
	Temperature limitation	**PCE**	Positive Control of Extraction
	Upper limit of temperature	**NCA**	Negative control of amplification
	Manufacturer	**C–**	Negative control of extraction
	Date of manufacture	**C+**	Positive control of amplification
EC REP	Authorised representative in the European Community	**IC**	Internal control

List of Changes Made in the Instruction Manual

VER	Location of changes	Essence of changes
12.11.10	Through the text	Records about PCR kit variant 200 are deleted
25.12.10 KM	Cover page	The phrase “For Professional Use Only” was added
	Intended use	The phrase “The results of PCR analysis are taken into account in complex diagnostics of disease” 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 open reagents was added
	Key to Symbols Used	The explanation of symbols was corrected
23.06.11 VV	Cover page, text	The name of Institution was changed to Federal Budget Institution of Science “Central Research Institute for Epidemiology”