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For Professional Use Only

AmpliSens® *Streptococcus* spp.-EPh

PCR kit

Instruction Manual

AmpliSens®

EC REP

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1. INTENDED USE

AmpliSens® *Streptococcus* spp.-EPh PCR kit is an *in vitro* nucleic acid amplification test for qualitative detection of *Streptococcus* species DNAs in the clinical material (cerebrospinal fluid) and cell culture 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

Streptococcus species detection by the polymerase chain reaction (PCR) is based on the amplification of bacterial 16S ribosomal RNA gene using special primers. One of the primers is common for all bacteria while the other is specific for *Streptococcus* species. Selected primers allow detecting of all *Streptococci* including *S.pneumoniae, S.pyogenes, S.agalactiae, S.anginosus.* After PCR the amplified product is detected in agarose gel. **AmpliSens® *Streptococcus* spp.-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 wax layer. Wax melting and reaction mix components occur only at 95 °C.

3. CONTENT

AmpliSens® *Streptococcus* spp.-EPh PCR kit is produced in 2 forms:

AmpliSens® *Streptococcus* spp.-EPh PCR kit variant 50 R (0.5-ml tubes),

REF B18-50-R0,5-CE.

AmpliSens® *Streptococcus* spp.-EPh PCR kit variant 50 R (0.2-ml tubes),

REF B18-50-R0,2-CE.

AmpliSens® *Streptococcus* spp.-EPh PCR kit variant 50 R includes:

Reagent	*Description*	*Volume, ml*	*Amount*
PCR-mix-1-R *Streptococcus* spp. 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 red	red clear liquid	0.6	1 tube
Mineral oil for PCR	colorless viscous liquid	2.0	1 dropper bottle
Positive Control DNA *Streptococcus pneumoniae* (C+$_{S.pneumoniae}$)	colorless clear liquid	0.1	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 (see DNA-sorb-AM, **REF** K1-12-50-CE protocol).

AmpliSens® *Streptococcus* spp.-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).
- Vortex mixer.
- PCR box.
- Tube racks.
- Personal thermocyclers (for example, Palm-Cycler (Corbett Research, Australia), GeneAmp PCR System 2400, GeneAmp PCR System 2700 (Applied Biosystems, USA), MiniCycler, PTC-100 (MJ Research, USA), Terzik (DNA-Technology, Russia), Omn-E (ThermoHybaid)).
- 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

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

AmpliSens® ***Streptococcus*** **spp.-EPh** PCR kit is intended for analysis of DNA extracted with DNA extraction kits from:

- *Cerebrospinal fluid.*

- *Cell culture.*

6.1 *Cerebrospinal fluid* sample is obtained by lumbar puncture procedure. Only disposable needles and tubes should be used.

Deliver the sample within 1 day in a container with an icepack.

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

7. WORKING CONDITIONS

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

8. PROTOCOL

8.1. DNA Extraction

It's recommended that the following nucleic acid extraction kit is used:

- DNA-sorb-AM, **REF** K1-12-50-CE.

Carry out the DNA extraction in accordance with the manufacturer protocol.

Positive Control DNA *Streptococcus pneumoniae* ($C+_{S.pneumoniae}$) must be used during DNA extraction procedure. Add 10 µl of Positive control DNA *Streptococcus pneumoniae* ($C+_{S.pneumoniae}$) and 90 µl of Negative Control (C-) in the tube labeled PCE (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. Prepare the required number of the PCR tubes with **PCR-mix-1-R *Streptococcus* spp.** for amplification of DNA from clinical and control samples.
2. Add **10 µl** of **PCR-mix-2 red** 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 *Streptococcus* spp.
3. Add above 1 drop of **mineral oil for PCR** (about 25 µl). When using thermocycler with heating cover this step could be omitted.
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).

$C+_{S.pneumoniae}$ - Add 10 µl of **Positive Control DNA *Streptococcus pneumoniae*** to the tube labeled $C+_{S.pneumoniae}$ (Positive Control of Amplification).

8.2.2. 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 button to continue.

It is recommended to sediment drops from walls of tubes by short vortexing (1–3 s) before insertion them in a thermocycler.

Table 1

Amplification program for *Streptococcus* spp. DNA

	Thermocyclers with active temperature adjustment						Thermocyclers with block temperature adjustment		
	GeneAmp PCR System 2400 (Perkin Elmer), Omn-E (ThermoHybaid), Terzik (DNA-Technology)			**GeneAmp PCR System 2700 (Applied Biosystems), Palm-Cycler (Corbett Research)**			**PTC-100, MiniCycler (MJ Research)**		
Step	Tempe-rature	Time	Cycles	Tempe-rature	Time	Cycles	Tempe-rature	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 s	42	**95 °C**	10 s	42	**95 °C**	1 min	42
	65 °C	10 s		**65 °C**	25 s		**65 °C**	1 min	
	72 °C	10 s		**72 °C**	25 s		**72 °C**	1 min	
3	**72 °C**	1 min	1	**72 °C**	1 min	1	**72 °C**	1 min	1
4	**10 °C**	storage		**10 °C**	storage		**10 °C**	storage	

Amplification in thermocycler with block temperature adjustment lasts 2 h 30 min, in thermocycler with active temperature adjustment — 1 h 50 min.

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 than the following detection agarose kit is used:

- 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 fragment is:

- *Streptococcus* spp.- 763 bp

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

9.1. Results interpretation

Table 2

Results for controls

Control	Step for control	Specific bands in the agarose gel 763 bp	Interpretation
PCE	DNA extraction	Yes	OK
C-	DNA extraction	No	OK
NCA	Amplification	No	OK
C+	Amplification	Yes	OK

- The sample is considered to be positive for *Streptococcus* spp. DNA if the band of 763 bp is present in agarose gel.
- The sample is considered to be negative for *Streptococcus* spp. DNA if the band of 763 bp is absent.

Besides the 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

Analysis results are not obtained as per the following examples:

- If results of control points analysis do not correspond to the listed above (Table 2), then the tests are to be re-installed. Discard any reagents that may be suspect.
- If in lane corresponding to positive control (C+) band of 763 nucleotide pairs is not observed, result of analysis is irrelevant. It can be caused by mistake in PCR conducting or amplification program fault.
- 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 lane corresponding to negative control (NCA, C–) specific band of 763 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 if encounter problems, please contact our Authorized representative in the European Community.

11. TRANSPORTATION

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

12. STABILITY AND STORAGE

All components of **AmpliSens®** ***Streptococcus*** **spp.-EPh** PCR kit are to be stored at 2–8 ºC when not in use. All components of the PCR kit are to be stable until the 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 **AmpliSens®** ***Streptococcus*** **spp.-EPh** PCR kit is no less than $5x10^3$ genome equivalents per 1 ml of sample (GE/ml).

The claimed analytical features of **AmpliSens®** ***Streptococcus*** **spp.-EPh** PCR kit are guaranteed only when additional kits of reagents, DNA-sorb-AM and EPh (manufactured by Federal Budget Institute of Science "Central Research Institute for Epidemiology"), are used.

13.2. Specificity

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

14. REFERENCES

1. Gillespie SH. The role of the molecular laboratory in the investigation of Streptococcus pneumoniae infections. Semin Respir Infect. 1999 Sep;14(3):269-75.
2. Manual "Sampling, transportation and storage of clinical material for PCR diagnostics", developed by Federal Budget Institute of Science "Central Research Institute for Epidemiology", Moscow, 2008.

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 **AmpliSens®** ***Streptococcus spp.*****-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	**NCA**	Negative control of amplification
	Manufacturer	**C–**	Negative control of extraction
	Date of manufacture	**C+**$_{S.pneumoniae}$	Positive Control of *S.pneumoniae*
EC REP	Authorised representative in the European Community	**PCE**	Positive control of Extraction

List of Changes Made in the Instruction Manual

VER	Location of changes	Essence of changes
24.12.10 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
28.06.11 VV	Cover page, text	The name of Institute was changed to Federal Budget Institute of Science “Central Research Institute for Epidemiology”