



For Professional Use Only

AmpliSens[®] *Corynebacterium diphtheriae*-EPh
PCR kit
Instruction Manual

AmpliSens[®]



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TABLE OF CONTENTS

1. INTENDED USE.....	3
2. PRINCIPLE OF PCR DETECTION	3
3. CONTENT	3
4. ADDITIONALLY REQUIRED MATERIALS, REAGENTS AND DEVICES.....	4
5. GENERAL PRECAUTIONS	4
6. SAMPLING AND HANDLING	5
7. WORKING CONDITIONS	5
8. PROTOCOL	5
9. DATA ANALYSIS	7
10. TROUBLESHOOTING	7
11. TRANSPORTATION	8
12. STABILITY AND STORAGE	8
13. SPECIFICATIONS	8
14. REFERENCES.....	8
15. QUALITY CONTROL.....	9
16. KEY TO SYMBOLS USED	10

1. INTENDED USE

AmpliSens® *Corynebacterium diphtheriae*-EPh PCR kit is an *in vitro* nucleic acid amplification test for qualitative detection of *Corynebacterium diphtheriae* toxigenic strains 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

Corynebacterium diphtheriae by the polymerase chain reaction (PCR) is based on the amplification of pathogen DNA specific region using special *Corynebacterium diphtheriae* primers. After PCR the amplified product is detected in agarose gel. **AmpliSens® *Corynebacterium diphtheriae*-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® *Corynebacterium diphtheriae*-EPh PCR kit is produced in 2 forms:

AmpliSens® *Corynebacterium diphtheriae*-EPh PCR kit variant 50 R (tubes of 0.5 ml volume), **REF** B8-50-R0,5-CE.

AmpliSens® *Corynebacterium diphtheriae*-EPh PCR kit variant 50 R (tubes of 0.2 ml volume), **REF** B8-50-R0,2-CE.

AmpliSens® *Corynebacterium diphtheriae* -EPh PCR kit variant 50 R includes:

Reagent	Description	Volume, ml	Quantity
PCR-mix-1-R <i>Corynebacterium diphtheriae</i> ready-to-use single-dose test tubes (<i>under wax</i>)	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 tube
Positive Control DNA <i>Corynebacterium diphtheriae</i> tox+ (C+ <i>Cor.dip.tox+</i>)	colorless clear liquid	0.1	1 tube
DNA-buffer	colorless clear liquid	0.5	1 tube
Negative Control (C-)*	straw-colored clear liquid	1.6	1 tube

* must be used in the extraction procedure as Negative Control of Extraction (see

DNA-sorb-B, **REF** K1-2-100-CE protocol)

AmpliSens® *Corynebacterium diphtheriae*-EPh PCR kit variant 50 R is intended for 55 reactions, including controls.

4. ADDITIONALLY REQUIRED MATERIALS, REAGENTS AND DEVICES

- 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
- Tube racks.
- PCR box
- Personal thermocycler (for example, GeneAmp PCR System 2400 (Perkin Elmer, USA) 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



Obtaining samples of biological materials for PCR-analysis, transportation and storage are described in manufacturer's handbook [1]. It is recommended to read this handbook before starting work

AmpliSens® *Corynebacterium diphtheriae*-EPh PCR kit is intended for analysis of DNA extracted by DNA extraction kit from the clinical material

7. WORKING CONDITIONS

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

8. PROTOCOL

8.1. DNA Extracion

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

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



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



Positive and Internal controls of extraction are not used

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 required quantity of the PCR tubes with **PCR-mix-1-R *Corynebacterium diphtheriae*** and wax for amplification of DNA from clinical and control samples.
2. Add **10 µl of PCR-mix-2 blue** to the surface of wax layer, ensuring that it doesn't fall under the wax and mix with **PCR-mix-1-R *Corynebacterium diphtheriae***.
3. Add above 1 drop of **mineral oil for PCR** (about 25 µl).

8.2.2 Amplification

1. Use prepared tubes for PCR. Under or immediately above the level of oil, using tips with aerosol barrier, **add 10 µl of DNA samples**, obtained from clinical or control samples at the stage of DNA extraction.
2. Perform **control amplification reactions**:
 - NCA - Add 10 µl of **DNA-buffer** to the tube for Negative Control of Amplification (NCA).
 - C+ - Add 10 µl of **Positive Control DNA *Corynebacterium diphtheriae* tox+** to the tube for Positive Control of Amplification
3. 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 vortex (1–3 s) before their insertion in thermocycler.

Table 1

Amplification program of *Corynebacterium diphtheriae*

Step	Thermocyclers with active temperature adjustment:			Thermocyclers with block temperature adjustment:		
	GeneAmp PCR System 2400 (Perkin Elmer), Omn-E (Hibaid)			MiniCycler, PTC-100 (MJ Research)		
	Temperature	Time	Cycles	Temperature	Time	Cycles
0	95 °C	pause		95 °C	pause	
1	95 °C	2 min	1	95 °C	2 min	1
2	95 °C	10 s	42	95 °C	1 min	42
	65 °C	10 s		65 °C	1 min	
	72 °C	10 s		72 °C	1 min	
3	72 °C	1 min	1	72 °C	1 min	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:

- *Corynebacterium diphtheriae* - 360 bp



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

Results interpretation

Table 2

Results for controls

Control	Which step of test is controlled	Specific bands in the agarose gel 360 bp	Interpretation
C-	DNA extraction	No	OK
NCA	Amplification	No	OK
C+	Amplification	Yes	OK

- The sample is considered to be positive for *Corynebacterium diphtheriae* DNA if the band of 360 bp is present in agarose gel.
- The sample is considered to be negative for *Corynebacterium diphtheriae* DNA if the band of 360 bp is absent.

Besides specific band 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.
- If in lane corresponding to positive control (C+) band of 360 nucleotide pairs is not observed, 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 lanes corresponding to negative control (NCA, C–) specific band of 360 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® *Corynebacterium diphtheriae*-EPh PCR kit should be transported at 2–8 °C for no longer than 5 days.

12. STABILITY AND STORAGE

All components of the **AmpliSens® *Corynebacterium diphtheriae*-EPh** PCR kit are to be stored at 2-8 °C 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.

13. SPECIFICATIONS

13.1. Sensitivity

Analytical Sensitivity of **AmpliSens® *Corynebacterium diphtheriae*-EPh** PCR kit is no less than 1×10^3 bacterial cells per 1 ml of sample (cells/ml).



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

13.2. Specificity

Specificity of **AmpliSens® *Corynebacterium diphtheriae*-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 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® *Corynebacterium diphtheriae*-EPh** PCR kit is tested against predetermined specifications to ensure consistent product quality.

16. KEY TO SYMBOLS USED

	Catalogue number		Sufficient for
	Batch code		Expiration Date
	<i>In vitro</i> diagnostic medical device		Consult instructions for use
	Version		Keep away from sunlight
	Temperature limitation	NCA	Negative control of amplification
	Manufacturer	C-	Negative control of extraction
	Date of manufacture	C+	Positive control of amplification
	Authorised representative in the European Community	IC	Internal control
	Caution		

List of Changes Made in the Instruction Manual

VER	Location of changes	Essence of changes
23.12.10 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
17.06.11 VV	Cover page, text	The name of Institute was changed to Federal Budget Institute of Science "Central Research Institute for Epidemiology"