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For Professional Use Only

AmpliSens® *Adenovirus*-EPh PCR kit

Instruction Manual

AmpliSens®

EC REP

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1. INTENDED USE

AmpliSens® *Adenovirus*-EPh PCR kit is an in vitro nucleic acid amplification test for qualitative detection of *Adenovirus* DNA in the clinical material (feces, oropharyngeal swabs (washing fluid), and eye discharge) 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

Adenovirus detection by the polymerase chain reaction (PCR) is based on the amplification of the pathogen cDNA specific region using specific *Adenovirus* primers. After PCR, the amplified product is detected in agarose gel. **AmpliSens® *Adenovirus*-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

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

AmpliSens® *Adenovirus*-EPh PCR kit variant 50 R (0.5-ml tubes), **REF** V23-50-R0,5-CE.

AmpliSens® *Adenovirus*-EPh PCR kit variant 50 R (0.2-ml tubes), **REF** V23-50-R0,2-CE.

AmpliSens® *Adenovirus* -EPh PCR kit variant 50 R:

Reagent	***Description***	***Variant 50 R***	
		Volume (ml)	***Amount***
PCR-mix-1-R *Adenovirus* 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 *Adenovirus* (C+ *Adenovirus*)	colorless clear liquid	0.1	1 tube
DNA-buffer	colorless clear liquid	0.5	1 tube
Negative Control (C–)*	colorless clear liquid	1.2	3 tubes

* must be used in the isolation procedure as Negative Control of Extraction (see DNA-sorb-B, **REF** K1-2-50-CE protocol).

AmpliSens® *Adenovirus*-EPh PCR kit variant 50 R is intended for 55 reactions, including

controls.

4. ADDITIONAL REQUIREMENTS

- DNA isolation 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.
- Desktop centrifuge with rotor for 2 ml reaction tubes.
- PCR box.
- Personal thermocyclers (for example, Gradient Palm Cycler (Corbett Research, Australia), GeneAmp PCR System 2400, GeneAmp PCR System 2700 (Applied Biosystems), MiniCycler, PTC-100 (MJ Research), Terzik (DNA-Technology), Omn-E (ThermoHybaid), MaxyGene (Axygen).
- Disposable polypropylene microtubes for PCR (0.5- or 0.2-ml; 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 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 accordance with appropriate biosafety practices.
- Clean and disinfect all sample or reagent spills using a disinfectant, such as 0.5% sodium

hypochlorite or another suitable disinfectant.

- Avoid contact with the skin, eyes, and mucosa. If skin, eyes, or mucosa contact, immediately flush with water and seek medical attention.
- Material Safety Data Sheets (MSDS) are available on request.
- Use of this product should be limited to personnel trained in DNA amplification techniques.
- The laboratory process must be one directional, it should begin in the Extraction Area move to the Amplification and Detection Areas. 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® *Adenovirus*-EPh PCR kit is intended for analysis of DNA extracted with DNA isolation kits from the clinical material:

- *Feces;*
- *Eye discharge;*
- *Oropharyngeal swabs (washing fluids).*

6.1. *Fecal sample* (0.4–1.0 g) from a disposable plastic sachet or plastic container placed into a chamber-pot or bedpan or from diaper in infants should be transferred to a special sterile container.

Deliver the fecal specimen to a lab within 1 day in a container with an icepack.

6.1.1 *Preparation of 10-20 % fecal suspension* (omit in case of liquid feces).

1. Collect tubes with tightly sealed cap and pipette 4 ml of saline solution.
2. Transfer 0.4–1.0 g (0.4–0.1 ml) of fecal specimen with a spatula into prepared tubes. Stir well to ensure homogenous suspension.

6.1.2. *Preparation of clarified fecal suspension.*

1. Spin the tube with prepared suspension or liquid feces at 3,000 rpm for 20 min.
2. Use the required volume of the supernatant for RNA extraction. The remaining specimen should be transferred to a disposable tube and stored frozen for further use.

6.2. *Eye discharge* should be taken with a sterile probe and placed into a tube containing transport medium. Vortex the tube and remove 100 µl of the specimen for DNA extraction.

6.3. Oropharyngeal washing fluids (swabs) should be obtained by sterile probe and placed into a tube containing transport medium. Vortex the tube and remove 100 µl of the specimen for DNA extraction.

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

7. WORKING CONDITIONS

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

8. PROTOCOL

8.1. DNA Isolation

It is recommended to use the following nucleic acid extraction kits:

- DNA-sorb-B **REF** K1-2-50-CE.

Extract DNA according to the manufacturer's instruction.

Add 50 µl of Negative Control directly to **each** sample/lysis mixture.

Volume of clinical sample for DNA extraction should be 50 µl.

Negative Control preparation: add 50 µl of Negative Control (C-) to the tube

Positive Control preparation: add 40 µl of Negative Control (C-) and 10 µl of Positive Control DNA Adenovirus to the tube

8.2. Preparing PCR

The total reaction volume is **25 µl**, the volume of DNA sample is **10 µl**.

8.2.1 Preparing tubes for PCR

1. Prepare the required number of PCR tubes with **PCR-mix-1-R *Adenovirus*** 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 *Adenovirus*.
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+ - Add 10 µl of **Positive Control DNA *Adenovirus*** to the tube labeled C+ (Positive

Control of Amplification).

8.2.2 Amplification

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

It is recommended to sediment drops from walls of tubes by short centrifugation (1–3 s) before inserting them in the thermocycler.

Table 1

Programming thermocyclers for *Adenovirus* DNA amplification

	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), Gradient Palm Cycler (Corbett Research), MaxyGene (Axygen)**			**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**	2 min	1	**95 °C**	2 min	1	**95 °C**	2 min	1
2	**95 °C**	10 s	42	**95 °C**	10 s	42	**95 °C**	1 min	42
	63 °C	10 s		**63 °C**	25 s		**63 °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 thermocyclers with block temperature adjustment lasts for 2 h; in thermocyclers with active temperature adjustment, for 1 h 30 min.

After the reaction is finished, PCR tubes must be collected and transferred to the room for PCR product analysis.

The amplification products are analyzed by separation of DNA fragments in agarose gel.

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

9. DATA ANALYSIS

It is 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:

- *Adenovirus* - 462 bp

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

9.1. Interpretation of results

Table 2

Results for controls

Control	Controlled step	Specific 462-bp bands in agarose gel	Interpretation
PC	DNA isolation	Yes	OK
C–	DNA isolation	No	OK
NCA	Amplification	No	OK
C+	Amplification	Yes	OK

- The sample is considered to be positive for *Adenovirus* DNA if the specific 462-bp band is present in agarose gel.
- The sample is considered to be negative for *Adenovirus* DNA if the specific 462-bp band is absent.

In addition to the specific bands, fuzzy bands corresponding to primer dimers may appear in lanes below the 100-bp level.

10. TROUBLESHOOTING

Results of analysis are not taken into account in the following cases:

- If results of control points of analysis do not correspond to those listed above (Table 2), then analysis should be repeated. Discard any reagents that may be suspect.
- If the 462-bp band is not observed in the lane corresponding to positive control (PCE, C+), the result of analysis is irrelevant. This may be caused by clinical processing errors that led to the loss of DNA or inhibition of PCR. In this case, analysis of this sample should be repeated starting from the DNA extraction stage.
- If nonspecific bands are presented at different levels, this may be caused by the lack of “hot start” or by an incorrect temperature profile in the thermocycler.
- The appearance of the specific 462-bp band in lanes corresponding to negative controls (NCA, C–) suggests contamination of reagents or samples. In such cases, the results of analysis are considered to be invalid. Analysis of all samples must be repeated and measures to detect and eliminate the source of contamination must be taken.

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

11. TRANSPORTATION

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

12. STABILITY AND STORAGE

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

The claimed analytical features of AmpliSens® *Adenovirus*-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® *Adenovirus*-EPh** PCR kit is ensured by selection of specific primers and strict reaction conditions as well as laboratory and clinical trials.

14. REFERENCES

1. Akhtar N, Ni J, Langston C, Demmler GJ, Towbin JA. PCR diagnosis of viral pneumonitis from fixed-lung tissue in children. Biochem Mol. Med. 1996 Jun;58(1):66-76.
2. Handbook “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 accordance with Federal Budget Institute of Science “Central Research Institute for Epidemiology” ISO 13485-Certified Quality Management System, each lot of **AmpliSens® *Adenovirus*-EPh** PCR kit is tested against predetermined specifications to ensure consistent product quality.

16. KEY TO SYMBOLS USED

REF	Catalogue number	Σ	Sufficient for
LOT	Batch code		Expiration Date
IVD	*In vitro* diagnostic medical device		Consult instructions for use
VER	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
EC REP	Authorised representative in the European Community	**IC**	Internal control
	Caution	**PC**	Positive Control of Extraction

List of Changes Made in the Instruction Manual

VER	Location of changes	Essence of changes
08.12.10	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
	Content	The volume of Negative Control was changed from 1.6 ml to 1.2 ml
14.06.11 VV	Cover page, text	The name of Institute was changed to Federal Budget Institute of Science “Central Research Institute for Epidemiology”