CE

IVD

For Professional Use Only

AmpliSens® *VZV*-FRT

PCR kit

Instruction Manual

AmpliSens®

EC REP

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1. INTENDED USE

AmpliSens® *VZV*-FRT PCR kit is an *in vitro* nucleic acid amplification test for qualitative detection of *Varicella-Zoster virus* DNA in clinical material (peripheral blood plasma, umbilical blood plasma, amniotic fluid, cerebrospinal fluid (CSF), blister content, saliva, oropharyngeal washes and swabs) by using real-time hybridization-fluorescence detection.

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

2. PRINCIPLE OF PCR DETECTION

Varicella-Zoster virus DNA detection by the polymerase chain reaction (PCR) is based on the amplification of the pathogen genome specific region using specific *VZV* primers. In real-time PCR, the amplified product is detected using fluorescent dyes. These dyes are linked to oligonucleotide probes which bind specifically to the amplified product during thermocycling. The real-time monitoring of fluorescence intensities during the real-time PCR allows the detection of accumulating product without re-opening the reaction tubes after the PCR run. **AmpliSens® *VZV*-FRT** PCR kit is a qualitative test that contains 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® *VZV*-FRT** 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 a chemically modified polymerase (TaqF). Chemically modified polymerase (TaqF) is activated by heating at 95 °C for 15 min.

3. CONTENT

AmpliSens® *VZV*-FRT PCR kit is produced in 1 form:

AmpliSens® ***VZV*-FRT** PCR kit variant FRT-50 F (for use with RG) **REF** R-V61-50-F(RG)-CE.

AmpliSens® *VZV*-FRT PCR kit variant FRT-50 F includes:

Reagent	*Description*	*Volume (ml)*	*Quantity*
PCR-mix-1-FL *VZV*	colorless clear liquid	0.6	1 tube
PCR-mix-2-FRT	colorless clear liquid	0.3	1 tube
Polymerase (TaqF)	colorless clear liquid	0.03	1 tube
Positive Control DNA *VZV*-FL ($C+_{VZV}$)	colorless clear liquid	0.1	1 tube
TE-buffer	colorless clear liquid	0.5	1 tube
Negative Control (C–)*	colorless clear liquid	0.5	2 tubes
Internal Control STI-87 (IC)**	colorless clear liquid	0.6	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 (RIBO-prep, **REF** K2-9-Et-50-CE).

AmpliSens® *VZV*-FL PCR kit is intended for 60 reactions (including controls).

4. ADDITIONAL REQUIREMENTS

- DNA/RNA extraction kit.
- Disposable powder-free gloves and laboratory coat.
- Pipettes (adjustable).
- Sterile pipette tips with aerosol barriers (up to 200 µl).
- Tube racks.
- Vortex mixer.
- Desktop centrifuge with a rotor for 2-ml reaction tubes.
- PCR box.
- Personal thermocyclers (for example, Rotor-Gene 3000 or Rotor-Gene 6000 (Corbett Research, Australia), Rotor-Gene Q (Qiagen, USA), or equivalent).
- Disposable polypropylene microtubes for PCR (0.1- 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, and 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 the DNA amplification techniques.
- The laboratory process must be one-directional, it should begin in the Extraction Area and then move to the Amplification and Detection Area. Do not return samples, equipment, and reagents to the area in which the previous step was performed.

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 that this handbook is read before starting work.

The clinical material must be taken according to state and local authorities' requirements.

7. WORKING CONDITIONS

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

8. PROTOCOL

8.1. RNA/DNA extraction

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

- RIBO-prep, **REF** K2-9-Et-50-CE.
- NucliSENS easyMAG automated system (BioMérieux) can also be used

Extract RNA/DNA according to the manufacturer's instructions.

8.2. Preparing PCR

8.2.1. Preparing tubes for PCR

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

1. Prepare the mixture of **PCR-mix-2-FRT** and **polymerase (TaqF):** transfer the entire content of the tube with **polymerase (TaqF) (30 µl)** into the tube with **PCR-mix-2-FRT (300 µl)** and carefully vortex. Mark the date of mixture's preparation on the tube.

This mixture is calculated for analysis of 60 samples.
Store the mixture at 2–8 °C for 3 months and use as necessary.

If the mixture can't be used within 3 months, prepare the mixture for less number of reactions, for example, mix 150 µl of PCR-mix-2-FRT and 15 µl of polymerase (TaqF) (for 30 reactions).

2. Prepare the reaction mixture. Keep in mind that the analysis of even one DNA sample should include two controls of amplification: positive control (Positive Control DNA *VZV*-FL ($C+_{VZV}$)) and negative control (TE-buffer). Moreover, when calculating reagent volumes add one extra reaction.
3. Mix **PCR-mix-1-FL *VZV*** and the **mixture of PCR-mix-2-FRT and polymerase (TaqF)** in a single tube. Volumes pre one PCR reaction are the following:
   - **10 µl of PCR-mix-1-FL *VZV***
   - **5 µl of mixture of PCR-mix-2-FRT and polymerase (TaqF)**

   Calculations of the reaction mixture for different number of reactions are provided in the Appendix 1.

When the total number of reactions is 60 use the simplified preparation:
transfer the entire content of the tubes with PCR-mix-1-FL *VZV* and polymerase (TaqF) into the tube with PCR-mix-2-FRT

4. Take the required number of tubes for amplification of DNA from clinical and control samples.
5. Transfer **15 µl** of the prepared reaction mixture to each PCR tube.
6. Add **10 µl** of **DNA samples** obtained from the clinical and control samples.
7. Carry out the control reactions:

$C+_{VZV}$ -Add **10 µl** of **Positive Control DNA *VZV*** to the tube labeled $C+_{VZV}$

(Positive Control of Amplification).

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

C- - Add **10 µl** of the **DNA sample** extracted from the Negative Control to the tube labeled C– (Negative Control of Extraction)

8.2.2. Amplification

Program the real-time amplification instrument according to manufacturer's manual.

1. Create a temperature profile on your instrument as follows:

Table 1

AmpliSens-1 amplification program for rotor-type instruments[1]

Step	*Temperature, °C*	*Time*	*Fluorescence detection*	Cycles
1	**95**	15 min	–	**1**
2	**95**	5 s	–	5
	60	20 s	–	
	72	15 s	–	
3	**95**	5 s	–	45
	60	20 s	FAM/Green, JOE/Yellow	
	72	15 s	–	

2. Adjust the fluorescence channel sensitivity according to *Important Product Information Bulletin.*
3. Insert tubes into the reaction module of the device.
4. Run the amplification program with fluorescence detection.
5. Analyze results after the amplification program is completed.

9. DATA ANALYSIS

– **Internal Control DNA** is detected in the **FAM/Green** fluorescence channel,

– ***Varicella-Zoster virus* DNA** is detected in the **JOE/Yellow** fluorescence channel.

See **Guidelines** for data analysis settings for the instrument.

The result of amplification in the appropriate channel is considered positive if a fluorescence curve is S-shaped (typical real-time PCR shape) and crosses the threshold line at the area of reliable growth of fluorescence.

The result of amplification in the appropriate channel is considered negative if a fluorescence curve does not have the typical shape and does not cross the threshold line (Ct is undefined).

9.1. Interpretation of results

– *VZV* DNA is **detected** in a sample if the Ct value defined in the JOE/Yellow channel does not exceed the boundary Ct value specified in the *Important product information bulletin.* Moreover, the fluorescence curve should cross the threshold line in the region

[1] For example,Rotor-Gene 3000, Rotor-Gene 6000, Rotor-Gene Q, or equivalent.

of fluorescence exponential growth.

– *VZV* DNA is **not detected in** a sample if the Ct value in the JOE/Yellow channel is not defined (absent) and the Ct value in the FAM/Green channel does not exceed the boundary Ct value specified in the *Important product information bulletin*.

– The result is **invalid** if the Ct value of a sample in the JOE/Yellow channel is absent whereas the Ct value in the FAM/Green channel is either absent or greater than the specified boundary Ct value. Repeat the PCR test for such a sample.

– The result is considered **equivocal** if the Ct value in the JOE/Yellow channel is greater than the specified boundary Ct value. PCR analysis of this sample should be performed in duplicate. If a reproducible positive Ct value is obtained, the result is considered positive. If the obtained Ct values are not reproduced in two repeats, the result is **equivocal**.

The result of the analysis is considered reliable only if the results obtained for Positive and Negative Controls of amplification as well as for the Negative Control of extraction are correct (Table 3).

Table 2

Results for controls

Control	Stage for control	Ct value in channel		Interpretation
		JOE/Yellow	FAM/Green	
C–	DNA extraction	Neg	Pos (< boundary Ct value)	**OK**
NCA	Amplification	Neg	Neg	**OK**
C+*VZV*	Amplification	Pos (< boundary Ct value)	Neg	**OK**

*For boundary Ct values, see the Important Product Information Bulletin.

10. TROUBLESHOOTING

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

- If the Ct for the Positive Control of amplification (C+$_{VZV}$) in the JOE/Yellow channel is absent or greater than the boundary Ct value, PCR and detection should be repeated for all samples in which *Varicella-Zoster virus* DNA was not detected.
- If the Ct value is detected for C– in the JOE/Yellow channel and/or for NCA in the FAM/Green and JOE/Yellow channels in the results grid, this indicates contamination of reagents or samples. In such cases, the results of analysis must be considered as invalid. Test analysis must be repeated and measures to detect and eliminate the source of contamination must be taken.
- If clinical samples do not show Ct value in the FAM/Green channel (internal control),

this indicates DNA extraction failure. Repeat the analysis for such samples starting from DNA extraction.

- If the Ct value in the FAM/Green channel (internal control) is greater than the specified boundary Ct value and the Ct value in the JOE/Yellow channel (*VZV*) is greater than the specified boundary Ct value as well, the sample should be analyzed once again starting from the DNA extraction stage. High Ct values can be caused by DNA loss during extraction or by the presence of inhibitors.

11. TRANSPORTATION

AmpliSens® *VZV*-FL PCR kit should be transported at 2–8 °C for no longer than 5 days.

12. STABILITY AND STORAGE

All components of the **AmpliSens® *VZV*-FRT** PCR kit (except for PCR-mix-1-FL *VZV,* polymerase (TaqF), and PCR-mix-2-FRT) are to be stored at 2–8 °C when not in use. All components of the **AmpliSens® *VZV*-FRT** PCR kit are stable until the expiration date on the label. The shelf life of opened reagents is the same as that of unopened reagents, unless otherwise stated.

PCR-mix-1-FL *VZV,* polymerase (TaqF), and PCR-mix-2-FRT
are to be stored at temperature from minus 24 to minus 16 °C when not in use.

PCR-mix-1-FL *VZV* is to be kept away from light.

13. SPECIFICATIONS

13.1. Sensitivity

The analytical sensitivity of **AmpliSens® *VZV*-FRT** PCR kit is the following:

Clinical material	**Nucleic acid extraction kit**	**Sensitivity,** copies/ml
Peripheral blood plasma, umbilical blood plasma amniotic fluid, CSF, blister content, saliva, oropharyngeal swab and washes	RIBO-prep	500

13.2. Specificity

The analytical specificity of **AmpliSens® *VZV*-FRT** PCR kit is ensured by selection of specific primers and probes as well as strict reaction conditions. The primers and probes were checked for possible homologies to all sequences deposited in gene banks by sequence comparison analysis. Nonspecific responses were not detected during testing of

the following viruses (*Epstein-Bar virus, human cytomegalovirus, human herpes virus* I and II, *human herpes virus* VI, *measles virus, rubella virus, parvovirus* B19), bacterial agents (*Streptococcus pyogenes*, *Staphylococcus aureus*, *Streptococcus agalactiae*, etc.), and *Toxoplasma gondii*.

The clinical specificity of **AmpliSens® *VZV*-FRT** PCR kit was confirmed in laboratory clinical trials.

14. REFERENCES

1. Handbook “Sampling, Transportation, and Storage of Clinical Material for PCR Diagnostics”, developed by Federal State Institute of Science “Central Research Institute of Epidemiology” of Federal Service for Surveillance on Consumers’ Rights Protection and Human Well-Being, Moscow, 2008.

15. QUALITY CONTROL

In compliance with Federal State Institute of Science “Central Research Institute of Epidemiology” ISO 13485-Certified Quality Management System, each lot of **AmpliSens® *VZV*-FRT** PCR kit has been 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
	Manufacturer	**NCA**	Negative control of amplification
	Date of manufacture	**C–**	Negative control of extraction
FBIS CRIE	Federal Budget Institute of Science “Central Research Institute for Epidemiology”	**C+$_{vzv}$**	Positive control of amplification
		IC	Internal control

List of Changes Made in the Instruction Manual

VER	Location of changes	Essence of changes
01.03.12. Ivl	Through the text	Umbilical blood plasma was added to the list of study materials; material names are given more correctly
	Specificity	The list of viruses that were used to confirm the analytical specificity of the kit was broadened
25.06.12 Ivl	Title page, Key to symbols used	Symbol IVD *in vitro* diagnostic medical device was changed to RUO research use only