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 the clinical material (peripheral blood plasma, umbilical blood plasma, amniotic fluid, cerebrospinal fluid (CSF), blister content, saliva, oropharyngeal washes and swabs) using 'real-time' fluorescence-hybridization 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 (Internal Control STI-87 (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. CONTENTS

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*	colourless clear liquid	0.6	1 tube
PCR-mix-2-FRT	colourless clear liquid	0.3	1 tube
Polymerase (TaqF)	colourless clear liquid	0.03	1 tube
Positive Control DNA *VZV*-FL ($C+_{VZV}$)	colourless clear liquid	0.1	1 tube
TE-buffer	colourless clear liquid	0.5	1 tube
Negative Control (C–)*	colourless clear liquid	0.5	2 tubes
Internal Control STI-87 (IC)**	colourless 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*-FRT 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 RNase-free pipette tips with aerosol filters (up to 200 µl).
- Tube racks.
- Vortex mixer.
- Desktop centrifuge with a rotor for 2-ml reaction tubes.
- PCR box.
- Real-time instruments (for example, Rotor-Gene 3000, Rotor-Gene 6000 (Corbett Research, Australia); Rotor-Gene Q (QIAGEN, Germany)).
- Disposable polypropylene PCR tubes (0.1- or 0.2-ml) when working with PCR kit variant FRT-100 F:
    a) 0.2-ml PCR tubes with optical transparent domed or flat caps if a plate-type instrument is used;
    b) 0.2-ml PCR tubes with flat caps or strips of four 0.1-ml Rotor-Gene PCR tubes if a rotor-type instrument is used.
- Refrigerator for 2–8 °C.
- Deep-freezer with a temperature range from minus 24 to minus 16 °C.
- Reservoir for used tips.

5. GENERAL PRECAUTIONS

The user should always pay attention to the following:

- Use sterile pipette tips with aerosol barriers and use a new tip for every procedure.
- Store all extracted positive material (specimens, controls and amplicons) away from all other reagents and add it to the reaction mix in a distantly separated facility.
- Thaw all components thoroughly at room temperature before starting an assay.
- When thawed, mix the components and centrifuge briefly.
- Use protective gloves and laboratory cloths, 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 specimens and unused reagents in accordance with local regulations.
- Samples should be considered potentially infectious and handled in biological cabinet in compliance with appropriate biosafety practices.
- Clean and disinfect all specimens or reagents spills using a disinfectant, such as 0.5 % sodium hypochlorite or another suitable disinfectant.
- Avoid specimens and reagents contact with the skin, eyes, and mucous membranes. If these solutions come into contact, rinse the injured area immediately with water and seek medical advice immediately.
- Material Safety Data Sheets (MSDS) are available on request.
- Use of this product should be limited to personnel trained in DNA amplification techniques.
- Workflow in the laboratory must be one-directional, beginning in the Extraction Area and moving to the Amplification and Detection Area. Do not return samples, equipment and reagents in the area where 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 that this handbook is read before starting work.

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 (for details see Guidelines [2]).

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

If the mixture cannot be used within 3 months, prepare the mixture for a smaller 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 <u>two controls</u> of amplification: positive control (Positive Control DNA *VZV*-FL ($C+_{VZV}$)) and negative control (TE-buffer). Moreover, when calculating reagent volumes take into account 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 Table 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

Table 1

SCHEME OF REACTION MIXTURE PREPARATION

	Total reagent volume for the specified number of reactions	
Reagent volume per 1 reaction, µl	**10.0**	**5.0**
Number of clinical samples	**PCR-mix-1-FL *VZV**, µl**	**Mixture of PCR-mix-2-FRT and polymerase (TaqF)*, µl**
4	**70**	**35**
5	**80**	**40**
6	**90**	**45**
7	**100**	**50**
8	**110**	**55**
9	**120**	**60**
10	**130**	**65**
11	**140**	**70**
12	**150**	**75**
13	**160**	**80**
14	**170**	**85**
15	**180**	**90**
16	**190**	**95**
17	**200**	**100**
18	**210**	**105**
19	**220**	**110**
20	**230**	**115**
21	**240**	**120**
22	**250**	**125**
23	**260**	**130**
24	**270**	**135**
25	**280**	**140**
26	**290**	**145**
27	**300**	**150**
28	**310**	**155**
33	**360**	**180**

*The specified volumes include 2 control points (positive and negative control of amplification) and 1 extra reaction.

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 labelled C+$_{VZV}$ (Positive Control of Amplification).

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

C- Add **10 µl** of the **DNA sample** extracted from the Negative Control to the tube labelled 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 2

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	–	40
	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. Analyse results after the amplification program is completed.

9. DATA ANALYSIS

Analysis of results is performed by the software of the real-time PCR instrument used by measuring fluorescence signal accumulation in two channels:

- The signal of the IC DNA amplification product is detected in the channel for the FAM fluorophore.
- The signal of the ***Varicella-Zoster virus*** **DNA** amplification product is detected in the channel for the JOE fluorophore.

See Guidelines [2] for data analysis settings for the instrument.

The results are interpreted by the presence (or absence) of an intercept between the fluorescence curve and the threshold line set at the specific level that corresponds to the

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

presence (or absence) of a *Ct* value of the DNA sample in the corresponding column of the results grid.

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).

Principle of interpretation is the following:

- *VZV* DNA is **detected** in a sample if the Ct value determined in the results grid in the channel for the JOE fluorophore does not exceed the boundary *Ct* value specified in the *Important product information bulletin.* Moreover, the fluorescence curve of the sample should cross the threshold line in the area of typical exponential growth of fluorescence.
- *VZV* DNA is **not detected** in a sample if the *Ct* value is not determined (absent) in the channels for the JOE fluorophore, whereas the *Ct* value determined in the channel for the FAM fluorophore is less than the boundary *Ct* value specified in the *Important Product Information Bulletin*.
- The result is **invalid** if the *Ct* value is not determined (absent) in the channel for the JOE fluorophore, whereas the *Ct* value in the channel for the FAM fluorophore is not determined (absent) or is greater than the specified boundary *Ct* value. In such cases, the PCR analysis should be repeated starting from the DNA extraction stage. If the same result is obtained in the second run, re-sampling of material is recommended.
- The result is **equivocal** if the *Ct* value determined in the channel for the JOE fluorophore is greater than the boundary *Ct* value specified in the *Important Product Information Bulletin.* In such cases, the PCR analysis should be repeated starting from the DNA extraction stage. If the same result is obtained, the sample is considered positive. If the obtained *Ct* values are not reproduced in two repeats, the result is considered **equivocal**.

Boundary *Ct* values are specified in the *Important Product Information Bulletin* enclosed to the PCR kit. See also Guidelines [2]

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 3

Results for controls

Control	Stage for control	*Ct* value in channel		Interpretation
		JOE/Yellow	FAM/Green	
C–	DNA extraction	Absent	Pos (< boundary *Ct* value)	**OK**
NCA	Amplification	Absent	Absent	**OK**
C+$_{VZV}$	Amplification	Pos (< boundary *Ct* value)	Absent	**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* value determined for the Positive Control of amplification (C+$_{VZV}$) in the JOE/Yellow channel is absent or is 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 determined 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 (IC), 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.

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

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, 2010.
2. Guidelines to **AmpliSens® *VZV*-FRT** PCR kit for qualitative detection of *Varicella-Zoster virus* DNA in the clinical material (peripheral blood plasma, umbilical blood

plasma, amniotic fluid, cerebrospinal fluid (CSF), blister content, saliva, oropharyngeal washes and swabs) using ‘real-time’ fluorescence-hybridization detection.

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	Σ	Sufficient for
IVD	*In vitro* diagnostic medical device		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
EC REP	Authorised representative in the European Community	**C+$_{vzv}$**	Positive control of amplification
		IC	Internal control