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For Professional Use Only

# eSens VZV QL PCR kit

**REF ES3202B**

## Instructions for Use

## 1 INTENDED USE

**eSens VZV QL 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.

NOTE: The results of PCR analysis are to be 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.

**eSens VZV QL 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.

**eSens VZV QL PCR kit** uses "hot-start", which greatly reduces the frequency of nonspecifically primed reactions. "Hot-start" is guaranteed by using chemically modified polymerase (TaqF). Chemically modified polymerase (TaqF) is activated by heating at 95 °C for 15 min.

The PCR kit contains the system for prevention of contamination by amplicons using the enzyme uracil-DNA-glycosylase (UDG) and dUTP. The enzyme UDG recognizes and catalyzes the destruction of the DNA containing deoxyuridine, but has no effect on DNA containing deoxythymidine. Deoxyuridine is absent in the authentic DNA, but is always present in amplicons, because dUTP is a part of dNTP mixture in the reagents for the amplification. Due to the deoxyuridine containing contaminating amplicons are sensitive to the destruction by UDG before the DNA-target amplification. So the amplicons cannot be amplified.

The enzyme UDG is thermolabile. It is inactivated by heating at temperature above 50 °C. Therefore, UDG does not destroy the target amplicons which are accumulated during PCR.

The results of amplification are registered in the following fluorescence channels:

**Table 1**

| **Channel for fluorophore** | **FAM** | **JOE** |
|---|---|---|
| DNA-target | Internal Control DNA | *Human alphaherpesvirus* 3 DNA |
| Target gene | Artificially synthesized sequence | ORF38 DNA fragment |

# 3 CONTENT

**eSens VZV QL PCR kit (ES3202B)** includes:

| **Reagent** | **Description** | **Volume, ml** | **Quantity** |
|---|---|---|---|
| **PCR-mix-1-FL *VZV*** | clear liquid from colorless to light lilac colour | 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 STI-87 (IC)** during the DNA extraction procedure directly to the sample/lysis mixture.

**eSens VZV QL 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 Q (QIAGEN, Germany), CFX 96 Touch, CFX 96 Opus (Bio-Rad, USA), QuantStudio 5 (Thermo Fisher Scientific), or equivalent).
- Disposable polypropylene PCR tubes (0.1- or 0.2-ml):

- 0.2-ml PCR tubes with optical transparent domed or flat caps if a plate-type instrument is used;
- 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 filters 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 disposable 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 samples or reagents spills using a disinfectant, such as 0.5 % sodium hypochlorite or another suitable disinfectant.
- Avoid inhalation of vapors, samples and reagents contact with the skin, eyes, and mucous membranes. Harmful if swallowed. If these solutions come into contact, rinse the injured area immediately with water and seek medical advice if necessary.
- Safety Data Sheets (SDS) 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

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

**eSens VZV QL PCR kit** is intended for the analysis of DNA extracted with DNA extraction kits from the clinical material (peripheral blood plasma, umbilical blood plasma, amniotic fluid, cerebrospinal fluid (CSF), blister content, saliva, oropharyngeal washes and swabs).

# 7 WORKING CONDITIONS

**eSens VZV QL PCR kit** should be used at 18–25 °C.

# 8 PROTOCOL

## 8.1 RNA/DNA extraction

Ecoli Dx, s.r.o. recommends:

- For the manual extraction
  - **RIBO-prep** (K2-9-Et-100-CE)

- For the automatic extraction

  - **ePure Viral Nucleic acid Extraction Kit** (E2003).

NOTE: 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.

NOTE: 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 two controls 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 2.

NOTE: 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 2**

**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 3**

**eSens amplification program for rotor-type instruments (e.g Rotor-Gene Q or equivalent)**

| 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, JOE | |
| | 72 | 15 s | – | |

2. Adjust the fluorescence channel sensitivity.
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.

## 8.3 Instrument Settings

**Test settings for rotor-type instruments**

**Rotor-Gene 3000/6000/Q**

| Channel | Calibrate/Gain Optimisation | Threshold | Dynamic tube | Slope Correct | More Settings/ Outlier Removal |
|---|---|---|---|---|---|
| FAM/Green | from 5FI to 10FI | 0.03 | On | On | 10 % |
| JOE/Yellow | from 5FI to 10FI | 0.03 | On | On | 10 % |

# 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.

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 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 (see Tab.5). 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 (see Tab.5).
- 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 (see Tab.5). 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 (see Tab.5). 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**.

**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 (see Table 4).**

**Table 4**

**Results for controls**

| **Control** | **Stage for control** | ***Ct* value in the channel for fluorophore** | |
|---|---|---|---|
| | | **JOE/Yellow** | **FAM/Green** |
| **C–** | DNA extraction | Absent | < boundary *Ct* value |
| **NCA** | Amplification | Absent | Absent |
| **C+$_{VZV}$** | Amplification | < boundary *Ct* value | Absent |

**Table 5**

**Boundary *Ct* values**

| Sample | Channel | |
|---|---|---|
| | **FAM/Green** | **YOE/Yellow** |
| **C+** | absent | <23 |
| **C-** | <24 | absent |
| **NGA** | absent | absent |
| **Clinical samples** | <24 | <35 |

# 10 TROUBLESHOOTING

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

1. If the *Ct* value determined for the Positive Control of amplification ($C+_{VZV}$) in the channel for JOE fluorophore 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.
2. If the *Ct* value is determined for C– in the channel for JOE fluorophore and/or for NCA in the channels for FAM and JOE fluorophores, 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.
3. If *Ct* value is absent in the channel for FAM fluorophore for clinical samples, this indicates DNA extraction failure. Repeat the analysis for such samples starting from DNA extraction.
4. If the *Ct* value in the channel for FAM fluorophore is greater than the specified boundary *Ct* value, whereas the *Ct* value in the channel for JOE fluorophore is greater than the specified boundary *Ct* value, 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

**eSens VZV QL PCR kit** should be transported at 2–8 °C for no longer than 5 days.

# 12 STABILITY AND STORAGE

All components of the **eSens VZV QL 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 **eSens VZV QL 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.

NOTE: 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.

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

# 13 SPECIFICATIONS

## 13.1 Sensitivity

The analytical sensitivity of **eSens VZV QL PCR kit** is the following:

| Clinical material | Nucleic acid extraction kit | Sensitivity, copies/ml |
|---|---|---|
| Peripheral blood plasma, umbilical blood plasma<br>amniotic fluid,<br>CSF,<br>blister content,<br>saliva,<br>oropharyngeal swab and washes | RIBO-prep<br>ePure Viral Nucleic acid Extraction Kit | 500 |

### 13.2 Specificity

The analytical specificity of **eSens VZV QL 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 **eSens VZV QL PCR kit** was confirmed in laboratory clinical trials.

## 14 QUALITY CONTROL

The production process, including batch release, is carried out in accordance with an established quality management system certified according to ISO 13485.

**List of Changes Made in the Instruction Manual**

| VER | Location of changes | Essence of changes |
|---|---|---|
| 01_04/2022 | | |

KEY TO SYMBOLS USED

| | | | |
|---|---|---|---|
| REF | Catalogue number | Σ | Contains sufficient for <n> tests |
| LOT | Batch code | | Use-by Date |
| IVD | In vitro diagnostic medical device | | Consult instructions for use |
| VER | Version | | Keep away from sunlight |
| | Temperature limit | **NCA** | Negative control of amplification |
| | Manufacturer | **C-** | Negative control of extraction |
| | Date of manufacture | **$C+_{vzv}$** | Positive control of amplification |
| | Caution | **IC** | Internal control |

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