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eSens

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IVD

For Professional Use Only

eSens CCHFV QL PCR kit

REF ES3603B

Instructions for Use

1 INTENDED USE

eSens CCHFV QL PCR kit is an *in vitro* nucleic acid amplification test for qualitative detection of *Crimean-Congo hemorrhagic fever virus (CCHFV)* RNA in clinical material (blood plasma and serum) and ticks using real-time hybridization-fluorescence detection of amplified products.

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

2 PRINCIPLE OF PCR DETECTION

Crimean-Congo hemorrhagic fever virus detection includes:

1. RNA extraction from biological material sample;
2. Reverse transcription of RNA and amplification of *CCHFV* cDNA fragment with real-time hybridization-fluorescence detection.

CCHFV RNA detection by polymerase chain reaction (PCR) is based on the amplification of pathogen genome specific region using specific *CCHFV* primers. In the real-time PCR, the amplified product is detected with the use of 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 CCHFV QL PCR kit is a qualitative test that contains the Internal Control (Internal Control STI-87-rec (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 CCHFV QL 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 chemically modified polymerase (TaqF). The chemically modified polymerase (TaqF) is activated by heating at 95 °C for 15 min.

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

Table 1

Channel for fluorophore	FAM	JOE
DNA-target	Internal Control STI-87-rec (IC) cDNA	*CCHFV* cDNA
Target gene	Artificially synthesized sequence	L-gene

3 CONTENT

eSens CCHFV QL PCR kit (ES3603B) includes:

Reagent	Description	Volume, ml	Quantity
RT-PCR-mix-1-FRT *CCHFV*	clear liquid from colorless to light lilac colour	0.6	1 tube
RT-PCR-mix-2-FEP/FRT	colorless clear liquid	0.3	1 tube
RT-G-mix-2	colorless clear liquid	0.015	1 tube
Polymerase (TaqF)	colorless clear liquid	0.03	1 tube
TM-Revertase (MMlv)	colorless clear liquid	0.015	1 tube
Positive Control cDNA *CCHFV* / STI (C+$_{CCHFV\,/\,STI}$)	colorless clear liquid	0.1	1 tube
RNA-buffer	colorless clear liquid	0.6	2 tubes
Negative Control (C–)*	colorless clear liquid	1.6	1 tube
Positive Control *CCHFV*-FL-rec**	colorless clear liquid	0.03	5 tubes
Internal Control STI-87-rec (IC)***	colorless clear liquid	0.12	5 tubes
tRNA 1 µg/µl	colorless clear liquid	0.06	5 tubes

***** must be used in the extraction procedure as Negative Control of extraction (C–).

****** must be used in the extraction procedure as Positive Control of extraction (PCE).

******* add **10 µl** of **Internal Control STI-87-rec (IC)** during the RNA extraction procedure directly to the sample/lysis mixture.

eSens CCHFV QL PCR kit is intended for 60 reactions (including controls).

4 ADDITIONAL REQUIREMENTS

- 0.15 M sodium chloride or phosphate buffer solution (PBS) (sodium chloride, 137mM; potassium chloride, 2.7 mM; sodium monophosphate, 10 mM; potassium diphosphate, 2 mM, pH=7.5±0.2).
- RNA/DNA extraction kit.
- Homogenizer (for ticks homogenization).
- Stainless steel beads (7 mm diameter).
- Disposable powder-free gloves and a laboratory coat.
- Pipettes (adjustable).
- Sterile RNase-free pipette tips with 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:
  a) 0.2-ml PCR tubes with optical transparent domed 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 with the temperature range from 2 to 8 °C.
- Deep-freezer with the 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

eSens CCHFV QL PCR kit is intended for analysis of the RNA extracted with RNA/DNA extraction kit from clinical material (blood plasma and serum) and ticks:

6.1. *Blood plasma and blood serum*.

Take samples of peripheral blood after overnight fasting into the tube with 6% EDTA solution at a proportion of 1:20. Turn over the closed tube with a blood sample several times. Centrifuge the tube with a blood sample at 1600 g for 20 min to obtain a plasma sample. Collect a serum sample in accordance with the standard procedure. Take 100 µl of clinical material for the analysis.

6.2. *Ticks*.

Before tick pretreatment, pools of ticks should be formed. Each pool can contain 5-7 non-sated ticks, 2-3 ticks of semi-sated ticks, or 1 fully sated one. Use sterile porcelain mortars and sterile pestles for tick suspension preparation. If an automatic homogenizer TissueLyser LT is used, the following parameters are set for the ticks of *Hyalomma* genus: diameter of beads – 7 mm; frequency – 50 Hz/s; homogenization time – 12-15 min; buffer volume – 700 µl (non-sated ticks), 1,000-1,500 µl (sated ticks and pools). Prior to homogenization, sated ticks should be pierced with a sterile disposable needle to let blood out. Oily ticks should be washed with 70 % ethanol solution. Homogenize the ticks in 700 µl (if a sample consists of 1 non-sated tick) or 1-1.5 ml (for a tick pool or a sated tick) of 0.15 M sodium chloride or PBS buffer. Add the solution by small portions. Centrifuge the obtained suspension at 10,000 g for 1 min. Take 50 µl of the supernatant for RNA extraction with **RIBO-prep** reagent kit (K2-9-Et-100-CE).

Before analysis, the biological material can be stored at 2–8 °C for 1 day, at the temperature not more than minus 16 °C for 1 week. Ticks can be stored alive (up to 1 month) or at the temperature not more than minus 16 °C for 1 week and then at the temperature not more than minus 70 °C.

7 WORKING CONDITIONS

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

8 PROTOCOL

8.1 RNA extraction

Any commercial nucleic acid extraction kit, if IVD-CE validated for the indicated specimen types, could be used.

Ecoli Dx, s.r.o. recommends:

- For the manual extraction

- **RIBO-prep** (K2-9-Et-100-CE) – for RNA extraction from blood plasma and serum or suspension of non-sated or semi-sated ticks.

- For the automatic extraction

- **ePure Viral Nucleic Acid Extraction Kit** (E2003)

NOTE: Extract the RNA according to the manufacturer's protocol.
The DNA extraction for each sample is carried out in the presence of **Internal Control STI-87-rec**.

8.2 Preparing the tubes for reverse transcription and PCR

The type of tubes depends on the PCR instrument used for analysis. Use disposable filter tips for adding reagents, DNA and control samples into tubes.
The total reaction volume is **25 µl**, the volume of the RNA sample is **10 µl**.

1. Before starting work, thaw and thoroughly vortex all reagents of the kit. Make sure that there are no drops on the caps of the tubes.
2. Take the required number of PCR tubes for amplification of clinical and control samples (including two controls of extraction, PCE and C–, and two controls of reverse transcription and amplification, C+, and NCA). The type of tubes depends on the real-time PCR instrument used for the analysis.
3. Prepare the reaction mixture for the required number of reactions. To do this, mix in a new tube RT-PCR-mix-1-FRT *CCHFV*, RT-PCR-mix-2-FEP/FRT, polymerase (TaqF), TM-Revertase (MMlv), and RT-G-mix-2 in the following proportion (the calculation is specified for one reaction):

   10 µl of RT-PCR-mix-1-FRT CCHFV,
   5 µl of RT-PCR-mix-2-FEP/FRT,
   0.5 µl of polymerase (TaqF),
   0.25 µl of RT-G-mix-b,
   0.25 µl of TM-Revertase (MMlv).

4. Add **15 µl** of the prepared reaction mixture to each PCR tube.

NOTE: Do not store the prepared mixture.

5. Add **10 µl** of **RNA samples** extracted from the clinical and control samples to each PCR tube. Carefully mix by pipetting.
6. Run the **control reactions:**

NCA	–	Add **10 µl** of **RNA-buffer** to the tube labeled NCA (Negative Control of Amplification)
C+	–	Add **10 µl** of **Positive Control cDNA *CCHFV* / STI (C+$_{CCHFV\ /\ STI}$)** to the tube labeled C+ (Positive Control of Amplification)
NOTE:		Samples should be amplified immediately after mixing the reaction mixture with RNA samples and control samples.

8.2.1 Reverse transcription and amplification

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

Table 2

	Rotor-type instruments (e.g Rotor-Gene Q or equivalent)			**Plate-type instruments** (e.g CFX 96 Touch, CFX 96 Opus, QuantStudio 5 or equivalent.)		
Step	**Temperature°C**	**Time**	**Cycles**	**Temperature°C**	**Time**	**Cycles**
1	**50**	**30 min**	**1**	**50**	**30 min**	**1**
2	**95**	**15 min**	**1**	**95**	**15 min**	**1**
3	**95**	**10 s**	**5**	**95**	**10 s**	**5**
	54	**25 s**		**54**	**30 s**	
	72	**15 s**		**72**	**15 s**	
4	**95**	**10 s**	**45**	**95**	**10 s**	**45**
	50	**25 s Fluorescence acquiring**		**50**	**35 s Fluorescence acquiring**	
	72	**15 s**		**72**	**15 s**	

Fluorescent signal is detected in the channels for the **FAM** and **JOE** fluorophores.

2. Adjust the fluorescence channel sensitivity.
3. Insert the tubes into the reaction module of the device. **Well No. 1 should be loaded with a test tube.**
4. Run the amplification program with fluorescence detection.
5. Analyze results after the amplification program is completed.

8.3 Instrument Settings

Test settings for rotor-type instruments

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	0.05	On	On	10 %

Note: Set *NTC threshold* as 15 % if fluorescence curves in the FAM/Green and JOE/Yellow channels do not have exponential growth.

Test settings for plate-type instruments

Channel	Threshold fluorescence
FAM	In **Threshold fluorescence** menu, set the threshold line at a level where the fluorescence curves are linear. It is recommended to select the threshold line level equal to 200 for FAM channel and 500 for JOE/HEX channel. Normally, the threshold line should cross only the sigmoid curves of signal accumulation of positive samples and controls and should not cross the baseline; otherwise, the threshold level should be raised.
JOE/HEX	

9 DATA ANALYSIS

The result is interpreted by software of the used real-time instrument. The curves of fluorescence signal accumulation are analyzed in two channels:

- The signal of the IC cDNA amplification product is detected in the channel for the FAM fluorophore.
- The signal of the *CCHFV* cDNA amplification product is detected in the channel for the JOE fluorophore.

Results are interpreted by the crossing (or not-crossing) the fluorescence curve with the threshold line set at the specific level that corresponds to the presence (or absence) of a *Ct* value of the cDNA sample in the corresponding column of the results grid.

Principle of interpretation is the following:

- *CCHFV* cDNA is **detected** if *Ct* value determined in the channel for the JOE fluorophore does not exceed the specified boundary *Ct* value. Moreover, the fluorescence curve of the sample should cross the threshold line in the area of typical exponential growth of fluorescence.
- *CCHFV* cDNA is **not detected** if *Ct* value determined in the channel for the FAM fluorophore does not exceed the specified boundary *Ct* value, whereas *Ct* value in the channel for the JOE fluorophore is not determined or exceeds the specified boundary value.
- the result is **invalid** if *Ct* value in the channel for the JOE fluorophore is not determined (absent) and *Ct* value in the channel for the FAM fluorophore is not determined or exceeds the specified boundary value. In this case, PCR analysis of the required sample should be repeated beginning with the extraction.

The result of the analysis is considered reliable only if the results obtained for the Positive and Negative Controls of Amplification as well as for the Positive and Negative Controls of Extraction are correct (see Table 3 and Table 4).

Table 3

Results for controls

Control	Stage for control	*Ct* value in the channel for fluorophore	
		FAM	JOE
C–	RNA extraction	<boundary value	Absent
PCE	RNA extraction	<boundary value	<boundary value
NCA	Amplification	Absent	Absent
C+	Amplification	<boundary value	<boundary value

Table 4

Boundary *Ct* values

Sample	Rotor-type instruments		Plate-type instruments	
	Channel for fluorophore			
	FAM	JOE	FAM	JOE
	Detection of IC	Detection of *CCHFV*	Detection of IC	Detection of *CCHFV*
NCA	*Ct* is absent		*Ct* is absent	
C-	<28	*Ct* is absent	<31	*Ct* is absent
PCE	<28	<30	<31	<33
C+	<28	<28	<31	<31
Test samples	<28 (blood plasma/serum) <30 (tick suspension)	<38	<31(blood plasma/serum) <33 (tick suspension)	<39

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+) in the channel for the JOE fluorophore is absent or exceeds the boundary value, amplification of all samples in which *CCHFV* cDNA was not detected should be repeated once again.
2. If the *Ct* value determined for the Positive Control of extraction (PCE) in the channel for the JOE fluorophore is absent or exceeds the specified boundary value, extraction of all samples in which *CCHFV* cDNA was not detected should be repeated once again.
3. If the *Ct* value is determined for Negative Control of extraction (C–) in the channel for the JOE fluorophore PCR analysis should be repeated for all samples in which cDNA was detected in the channel for the JOE fluorophore.
4. If the *Ct* value is detected for Negative Control of amplification (NCA) in the channels for the FAM and JOE fluorophores, amplification of all samples in which cDNA was detected in the channel for the JOE fluorophore should be repeated once again accompanied with amplification of NCA sample in three replicates.

11 TRANSPORTATION

eSens CCHFV 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 CCHFV QL PCR kit** are to be stored at 2–8 °C when not in use (except for RT-G-mix-2, RT-PCR-mix-1-FRT *CCHFV,* RT-PCR-mix-2-FEP/FRT, polymerase (TaqF), TM-Revertase (MMlv), and tRNA 1µg/µl). All components of the **eSens CCHFV QL 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.

NOTE: RT-G-mix-2, RT-PCR-mix-1-FRT *CCHFV*, RT-PCR-mix-2-FEP/FRT, polymerase (TaqF), TM-Revertase (MMlv), and tRNA 1µg/µl are to be stored at temperature from minus 24 to minus 16 °C when not in use.

NOTE: RT-PCR-mix-1-FRT *CCHFV* is to be kept away from light.

13 SPECIFICATIONS

13.1 Sensitivity

The analytical sensitivity of **eSens CCHFV QL PCR kit** is specified in the table below.

Test material (sample volume)	RNA/DNA extraction kit	Analytical sensitivity, copies/ml	Pretreatment
Blood serum (100 µl)	RIBO-prep	$5x10^3$	The claimed sensitivity is achieved only when the material pretreatment is carried out in accordance with recommendation and the recommended volume of test sample is used.
H.marginatum tick pools (50 µl)			
H.marginatum ticks pools (100 µl)	ePure Viral Nucleic Acid Extraction Kit	$5x10^3$	

13.2 Specificity

The analytical specificity of **eSens CCHFV QL PCR kit** is ensured by selection of specific primers and probes and stringent reaction conditions. The primers and probes were checked for possible homologies to all sequences deposited in gene banks by sequence comparison analysis. Analytical specificity was examined with the use of the following microorganisms:

- *flaviviruses* (*West Nile fever virus*, *Omsk hemorrhagic fever virus*);
- *herpesviruses* (types I and II, *cytomegalovirus*; *Epstein-Barr virus*, *Varicella-Zoster virus*, type IV), *enteroviruses* (*ECHO*, *Coxsackie*);
- *rickettsiae* of the spotted fever group (*Rickettsia conorii* ssp. *caspia*, *Coxiella burnetii*);
- *orthobunyaviruses* (*Tyaginya virus*, *Batai virus*);
- *hantaviruses* (*Puumala virus*, *Dobrava virus*);
- *thogotoviruses* (*Batken virus*).

False positive results for the above-mentioned organisms and viruses as well as human DNA and tick DNA were not detected.
The clinical specificity of **eSens CCHFV 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.

15 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+**	Positive control of amplification
EC REP	Authorized representative in the European Community	**IC**	Internal control
⚠	Caution	**PCE**	Positive Control of extraction

List of Changes Made in the Instruction Manual

VER	Location of changes	Essence of changes
01_04/2022		

Ecoli Dx, s.r.o. , Purkyňova 74/2

110 00 Praha 1, Česká republika
Tel: +420 325 209 912

Mobil: +420 739 802 523