ECOLI

eSens

CE

IVD

For Professional Use Only

eSens EBOV Zaire QL PCR kit

REF ES3604B

Instructions for Use

1 INTENDED USE

eSens EBOV Zaire QL PCR kit is an *in vitro* nucleic acid amplification test for qualitative detection of the *Zaire Ebola virus* (*EBOV* Zaire) RNA in the biological material (whole blood, saliva, urine, viscera biopsy material), taken from the persons suspected of Ebola fever without distinction of form and presence of manifestation, 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

The method is based on the RNA extraction from the test material with the Internal Control (Internal Control STI-87-rec (IC)) and simultaneous RNA reverse transcription reaction and amplification of the cDNA fragments of the detected virus and Internal Control STI-87-rec cDNA with hybridization-fluorescence detection. The Internal Control must be used in the extraction procedure in order to control the extraction process of each individual sample and to identify possible reaction inhibition.

EBOV Zaire detection by the polymerase chain reaction (PCR) is based on the RNA reverse transcription using TM-Revertase enzyme and amplification of the pathogen genome specific region using specific *EBOV* Zaire 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 EBOV Zaire QL PCR kit uses “hot-start”, which greatly reduces the frequency of nonspecifically primed reactions. “Hot-start” is guaranteed by the separation of nucleotides and Taq-polymerase by using chemically modified polymerase (TaqF). The chemically modified polymerase (TaqF) is activated by heating at 95 °C for 15 min.

2 reactions are carried out in one tube at the RT-PCR stage – amplification of *EBOV* Zaire cDNA and Internal Control STI-87-rec cDNA sequence. The results of *EBOV* Zaire cDNA and Internal Control STI-87-rec cDNA amplification are registered in two different channels of fluorescence detection:

Table 1

Channel for fluorophore	FAM	JOE
cDNA-target	Internal Control STI-87-rec cDNA	*EBOV* Zaire cDNA
Target gene	Artificially synthesized sequence	L-gene

3 CONTENT

eSens EBOV Zaire QL PCR kit (ES3082A) includes:

Reagent	Description	Volume, ml	Quantity
PCR-mix-FL *EBOV* Zaire	clear liquid from colorless to light lilac colour	0.6	1 tube
PCR-buffer-C	colorless clear liquid	0.3	1 tube
Polymerase (TaqF)	colorless clear liquid	0.03	1 tube
TM-Revertase (MMlv)	colorless clear liquid	0.015	1 tube
RT-G-mix-2	colorless clear liquid	0.015	1 tube
Positive Control *EBOV* Zaire / STI (C+$_{EBOV\ Zaire\ /\ STI}$)	colorless clear liquid	0.2	1 tube
TE-buffer	colorless clear liquid	0.2	1 tube
Negative Control (C–)*	colorless clear liquid	1.2	1 tube
Internal Control STI-87-rec (IC)**	colorless clear liquid	0.5	1 tube

***** must be used in the extraction procedure as Negative Control of Extraction.

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

eSens EBOV Zaire QL PCR kit is intended for 55 reactions (including controls).

4 ADDITIONAL REQUIREMENTS

- Plastic container (50-60 ml) for storage and transportation of biological samples.
- Disposable polypropylene tightly closed tubes of 1.5 ml, 2.0 ml.
- Screw caps for the tubes.
- Vacuette blood collection system.
- Transport medium for urine samples pretreatment.
- 0.9 % sodium chloride solution (sterile physiological solution) or phosphate buffer solution (137 mM sodium chloride, 2.7 mM potassium chloride, 10 mM sodium monophosphate, 2 mM potassium diphosphate; pH=7.5±0.2).
- Sterile tools (individual for each sample) for homogenization (porcelain mortar and mallet) or homogenizer for pretreatment of viscera material.
- Vacuum aspirator with flask for removing supernatant.
- RNA extraction kit.

- Disposable powder-free gloves and a 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:
  a) 1.5-ml screwed or tightly closed tubes for reaction mixture preparation;
  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 at the temperature 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 EBOV Zaire QL PCR kit is intended for analysis of the RNA extracted with RNA extraction kits from the biological material (whole blood, saliva, urine, viscera biopsy material).

Sampling

6.1 Whole blood.

Blood samples are taken after overnight fasting or 3 hour fasting from median cubital vein by disposable needle (0.8-1.1 mm diameter) in a special Vacuette vacuum system (lilac caps with 6 % EDTA). After sampling a tube with blood is to be rotated gently several times for mixing with the anticoagulant (otherwise blood will coagulate and the RNA extraction will be impossible). Place the tube in a rack after mixing. The whole blood samples can be stored at room temperature for 2 hours, at 2- 8 °C for 12 hours.

6.2 Saliva.

Saliva samples are taken (after 3 mouthwashes with physiological solution) in the sterile dry tubes (2 ml) in an amount of at least 1 ml. Tightly cap the tube avoiding airspace and deformation of cap internal part. Mark the tube.
The saliva samples can be stored at room temperature for 6 hours, at 2-8 °C for 1 day or at the temperature from minus 24 to minus 16 °C for 1 week or at not more than minus 70 °C for a long time.

6.3 Urine.

The urine samples are taken in an amount of 15-25 ml into the dry sterile container (50-60 ml) or dry clean vessel.
The urine samples before pretreatment can be stored at 2-8 °C for 1 day or at the temperature from minus 24 to minus 16 °C for 1 week or at not more than minus 70 °C for a long time. Only one freeze-thaw cycle is allowed.

6.4 Viscera biopsy material.

Biopsy material is taken by a sterile tools in a sterile plastic containers with tightly closed caps or 2 ml tubes. The tube is to be closed tightly.
The biopsy material samples can be stored at room temperature for 6 hours, at 2-8 °C for 3 day or at the temperature from minus 24 to minus 16 °C for 1 week or at not more than minus 70 °C for a long time.

Pretreatment

6.5 Whole blood and *saliva samples*.

The pretreatment is not required.

6.6 Urine samples.

Urine samples are to be pretreated. Shake a vessel with urine. Transfer 1 ml of urine using a tip with filter in a 1.5 ml sterile disposable tube. Centrifuge at 10,000 g (for example, 12,000 rpm for microcentrifuge MiniSpin, Eppendorf Manufacturing Corporation) for 5 min. In the case of a large quantity of salts resuspend only the upper layer of salts sediment in a 1 ml volume and then concentrate again. Discard the supernatant completely using vacuum aspirator with flask and a new tip without filter for each sample and avoiding transferring the sediment. Add to the sediment the **Transport Medium with Mucolytic Agent** (952-CE) to the 0.2 ml final volume. Mix thoroughly by vortexing.
The pretreated urine sample can be stored at 2-8 °C for 1 day or at the temperature from minus 24 to minus 16 °C for 1 week or at not more than minus 70 °C for a long time.

6.7 Viscera biopsy material.

Viscera biopsy material is to be pretreated. For RNA extraction take 30-50 µl of the material and homogenize it by trituration using precooled sterile porcelain mortar and mallet or homogenizer. Prepare suspension using grinded tissue and precooled sterile physiological solution or phosphate buffer. For this, add 5 volumes of physiological solution to 1 volume of grinded tissue. Use 100 µl of suspension for RNA extraction.
The pretreated viscera biopsy material sample can be stored at the temperature from minus 24 to minus 16 °C for 1 week or at not more than minus 70 °C for a long time.

Interfering substances and limitations of using test material samples

The excessive amount of impurities in biological material such as mucus, blood, pus, and others can lead to the amplification reaction inhibition. In order to control the RNA extraction efficiency and possible reaction inhibition the Internal Control (Internal Control STI-87-rec (IC)) is used in the PCR kit. The Internal Control is added in each biological sample at the extraction stage. The presence of internal control signal after the amplification testifies the effectiveness of nucleic acid extraction and the absence of PCR inhibitors.
The next samples are inapplicable for analysis:

- the urine samples collected more than 24 hours before the delivery to the laboratory,
- the whole blood samples, collected in the tubes with heparin as anticoagulant,
- the whole blood samples, containing blood clot or which has been exposed to freezing.

7 WORKING CONDITIONS

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

8 PROTOCOL

8.1 DNA 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 the automatic extraction

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

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

8.2 Preparing reverse transcription and PCR

8.2.1 Preparing tubes for RT-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 RNA sample is **10 µl**.

1. Calculate the required quantity of each reagent for one reaction:

   10 µl of **PCR-mix-FL *EBOV* Zaire,**
   5 µl of **PCR-buffer-C,**
   0.5 µl of **Polymerase (TaqF),**
   0.25 µl of **TM-Revertase (MMlv),**
   0.25 µl of **RT-G-mix-2.**

Prepare the reaction mixture for required number of reactions (including clinical and control samples and one extra reaction).

NOTE: Prepare the reaction mixture just before use.

2. Thaw the tube with **PCR-mix-FL *EBOV* Zaire.** Thoroughly vortex all the reagents of the PCR kit and sediment the drops by vortex.
3. In a new tube prepare the reaction mixture. Mix the required quantities of **PCR-mix-FL *EBOV* Zaire, PCR-buffer-C, Polymerase (TaqF), TM-Revertase (MMlv) and RT-G- mix-2** in accordance to the table 2. Sediment the drops by vortex.

Table 2

Scheme of reaction mixture preparation

		Reagent volume for specified number of reactions				
Reagent volume per one reaction, µl		**10,00**	**5,00**	**0,25**	**0,50**	**0,25**
Number of test samples	**Number of reactions***	**PCR-mix-FL *EBOV* Zaire**	**PCR buffer-C**	**RT-G-mix-2**	**Polymerase (TaqF)**	**TM-Revertase (MMlv)**
1	**6**	**60**	**30**	**1.5**	**3.0**	**1.5**
3	**8**	**80**	**40**	**2.0**	**4.0**	**2.0**
5	**10**	**100**	**50**	**2.5**	**5.0**	**2.5**
7	**12**	**120**	**60**	**3.0**	**6.0**	**3.0**
9	**14**	**140**	**70**	**3.5**	**7.0**	**3.5**
11	**16**	**160**	**80**	**4.0**	**8.0**	**4.0**
13	**18**	**180**	**90**	**4.5**	**9.0**	**4.5**
15	**20**	**200**	**100**	**5.0**	**10.0**	**5.0**
17	**22**	**220**	**110**	**5.5**	**11.0**	**5.5**
19	**24**	**240**	**120**	**6.0**	**12.0**	**6.0**
21	**26**	**260**	**130**	**6.5**	**13.0**	**6.5**
23	**28**	**280**	**140**	**7.0**	**14.0**	**7.0**
25	**30**	**300**	**150**	**7.5**	**15.0**	**7.5**
27	**32**	**320**	**160**	**8.0**	**16.0**	**8.0**

***** Number of reactions = number of test samples + the controls of extraction stage and RT- PCR (C–, PCE, C+, NCA) + one extra reaction. (N+4+1, N - number of test samples).

4. Take the required number of the tubes or strips taking into account the number of test samples and control samples.
5. Transfer **15 µl** of the prepared reaction mixture to each tube. Discard the unused reaction mixture.
6. Add **10 µl** of **RNA samples** extracted from test or control samples of RNA extraction stage using tips with filter.

NOTE: Mix the tubes thoroughly by pipetting avoiding foaming.

7. Carry out the control reactions:

NCA	–	Add **10 µl** of **TE-buffer** to the tube labeled NCA (Negative Control of Amplification).
C+*EBOV Zaire* /STI	–	Add **10 µl** of **Positive Control *EBOV* Zaire / STI (C+*EBOV Zaire* / STI)** to the tube labeled **C+*EBOV Zaire* / STI** (Positive Control of Amplification).
C–	–	Add **10 µl** of **the sample extracted from the Negative Control (C–) reagent** to the tube labeled C– (Negative Control of Extraction).
PCE	–	Add **10 µl** of **the sample extracted from the Positive Control *EBOV* Zaire reagent** to the tube labeled PCE (Positive control of Extraction).
NOTE:		Mix the tubes thoroughly by pipetting avoiding foaming. Carry out the RT-PCR just after the mix of reaction mixture and RNA-samples and controls. Time of the addition of samples to the reaction mixture and the reaction run on the instrument cannot be more than 10-15 min.

8.2.2 Reverse transcription and amplification

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

Table 3

Amplification and detection program

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**	**Fluorescent signal detection**	**Cycles**
1	**50**	**30 min**	**–**	**1**
2	**95**	**15 min**	**–**	**1**
3	**95**	**10 s**	**–**	**5**
	55	**20 s**	**–**	
	72	**15 s**	**–**	
4	**95**	**10 s**	**–**	**40**
	55	**20 s**	**FAM, JOE**	
	72	**15 s**	**–**	

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

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. Analyze results after the amplification program is completed.

8.3 Instrument Settings

Test settings for rotor-type and plate-type instruments

Channel	**Calibrate/Gain Optimisation**	**Threshold**	**Dynamic tube**	**Slope Correct**	**More Settings/ Outlier Removal**
FAM/Green	from 5 to 10 FL	0.05	on	on	5 %
JOE/Yellow	from 5 to 10 FL	0.05	on	on	10-30 %

9 DATA ANALYSIS

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

Table 4

Channel for the fluorophore	FAM	JOE
Signal registration, indicating the amplification product accumulation	Internal Control STI-87- rec cDNA	*EBOV* Zaire cDNA

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

The results are interpreted in accordance with the Table 5.

Table 5

Results interpretation

***Ct* value in the channel for the fluorophore**		**Result**
FAM	**JOE**	
< boundary value	absent	*EBOV* Zaire RNA **is not detected**
determined or absent	< boundary value	*EBOV* Zaire RNA **is detected**
absent or > boundary value	absent or > boundary value	**Invalid** result Repeat the extraction and amplification

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 Positive and Negative Control of extraction are correct (see Table 6 and Table 7).

Table 6

Results for controls

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

Table 7

Boundary *Ct* values

Sample	Channel for the fluorophore	
	FAM/Green	**JOE/Yellow**
C+	35	36
NCA	Absent	Absent
C-	35	Absent
PCE	35	36
Test samples	35	38

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 greater than the boundary *Ct* value or absent, the amplification and detection should be repeated for all samples in which the *EBOV* Zaire RNA was not detected.
2. If the *Ct* value determined for the Positive Control of Extraction (PCE) in the channel for the **JOE** fluorophore is greater than the boundary *Ct* value or absent, the PCR analysis (beginning with the RNA extraction stage) should be repeated for all samples.
3. If the *Ct* value is determined for the Negative Control of Extraction (C–) in the channel for the **JOE** fluorophore, the contamination of laboratory with amplification fragments or contamination of reagents, test samples is probable at any stage of PCR analysis. Measures for detecting and elimination of contamination source must be taken. The PCR analysis (beginning with the RNA extraction stage) should be repeated for all samples in which *EBOV* Zaire RNA was detected.
4. If the *Ct* value is determined for the Negative Control of Amplification (NCA) in the channels for the **FAM** and/or **JOE** fluorophores, the contamination of laboratory with amplification fragments or contamination of reagents, test samples is probable at any stage of PCR analysis. Measures for detecting and elimination of contamination source must be taken. The amplification and detection should be repeated for all samples in which *EBOV* Zaire RNA was detected.

5. If the *Ct* value is determined for the test sample, whereas the area of typical exponential growth of fluorescence is absent (the graphic looks like approximate straight line). It is necessary to check that threshold line or parameters of threshold line measurement are correct. If the result has been obtained with the correct threshold line level, the amplification and detection should be repeated for this sample.

11 TRANSPORTATION

eSens EBOV Zaire 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 EBOV Zaire QL PCR kit** are to be stored at 2–8 °C when not in use (except for PCR-mix-FL *EBOV* Zaire, PCR-buffer-C, RT-G-mix-2, polymerase (TaqF), TM-Revertase (MMlv)). All components of the **eSens EBOV Zaire QL PCR kit** are stable until the expiry date stated on the label. The shelf life of reagents before and after the first use is the same, unless otherwise stated.

NOTE: PCR-mix-FL *EBOV Zaire*, PCR-buffer-C, RT-G-mix-2, polymerase (TaqF) and TM-Revertase (MMlv) are to be stored at the temperature from minus 24 to minus 16 °C

NOTE: PCR-mix-FL *EBOV* Zaire is to be kept away from light

13 SPECIFICATIONS

13.1 Analytical sensitivity

Biological material	Nucleic acid extraction kit	PCR kit	Sensitivity, GE/ml*
Whole blood, saliva, urine, viscera biopsy material	RIBO-prep	ES3082A	$2x10^3$
	ePure Viral Nucleic Acid Extraction Kit	ES3082A	$2x10^3$

***** Genome equivalents (GE) of the pathogen agent per 1 ml of a sample.

13.2 Analytical specificity

The analytical specificity of **eSens EBOV Zaire QL PCR kit** is ensured by selection of specific primers and probes as well as stringent reaction conditions. The primers and probes were checked for possible homologies to all sequences published in gene banks by sequence comparison analysis.

The specificity was proved on the follows viruses and strains of microorganisms: *Tahyna virus, Batai virus, Inkoo virus, CCHFV, Dhori virus, Yellow fever virus, WNV, Sindbis virus, Chikungunya virus, Rubella virus, Kemerovo virus, Rotavirus, Enteric Cytopathic Human Orphan virus, HIV, Rabies virus, CMV, Human parvovirus B19, Francisella tularensis*, *Yersinia enterocolitica, Yersinia pestis,* and human DNA. Nonspecific responses were absent in tests of DNA samples of this organisms and human DNA samples.

The clinical specificity of **eSens EBOV Zaire QL PCR kit** was confirmed in laboratory clinical trials.

13.3 Reproducibility and repeatability

The biological material with the addition of Ebola fever virus standard (manufactured by Robert-Koch-Institut, Berlin, Germany) with concentration 10^5 GE/ml was used as positive samples.

Biological material	Number of repeats	Coefficient of variation CV, %
Dispersion of values in a single test		
Whole blood	6	1.68
Saliva	6	0.64
Urine	6	1.11
Viscera biopsy material	6	0.74
Dispersion of values between tests, carried out in different days		
Whole blood	12	3.57
Saliva	12	1.13
Urine	12	1.09
Viscera biopsy material	12	0.89

13.4 Diagnostic characteristics

Results of eSens EBOV Zaire QL PCR kit testing in comparison with the reference assay

Sample type	Results of using eSens EBOV Zaire QL PCR kit		Results of using reference assay*	
			positive	negative
Whole blood	100 samples was analyzed	**positive**	48	0
		negative	0	52
Saliva	100 samples was analyzed	**positive**	29	0
		negative	0	71
Viscera biopsy material	100 samples was analyzed	**positive**	34	0
		negative	0	66
Urine	100 samples was analyzed	**positive**	43	0
		negative	0	57

***** RealStar Filovirus Screen RT-PCR Kit 1.0 diagnostic system for Ebola and Marburg viruses RNA detection by real-time PCR (Altona Diagnostics GmbH, Germany) was used as a reference assay.

Diagnostic characteristics of eSens EBOV Zaire QL PCR kit

Sample type	Diagnostic sensitivity4 (with a confidence coefficient of 90 %), no less than %	Diagnostic specificity5 (with a confidence coefficient of 90 %), no less than %
Whole blood	95	95
Saliva	93	97
Viscera biopsy material	93	96
Urine	94	95

14 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		Caution
LOT	Batch code		Contains sufficient for <n> tests
IVD	*In vitro* diagnostic medical device		Use-by Date
VER	Version		Consult instructions for use
	Temperature limit		Keep away from sunlight
	Manufacturer	NCA	Negative control of amplification
	Date of manufacture	C-	Negative control of extraction
EC REP	Authorized representative in the European Community	C+*EBOV Zaire* / STI	Positive control of amplification
PCE	Positive control of extraction	IC	Internal control

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