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eSens

CE

IVD

For Professional Use Only

eSens EBV/HHV6 QT PCR kit
PCR kit

REF ES3240A

Instructions for Use

1 INTENDED USE

eSens EBV/HHV6 QT PCR kit is an *in vitro* nucleic acid amplification test for qualitative and quantitative detection of *Epstein-Barr virus* (*EBV*) DNA and *Human Herpes virus* type 6 (*HHV*6) DNA in clinical material (whole blood, white blood cells, viscera biopsy material and cerebrospinal fluid) 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

Principle of testing is based on the DNA extraction from the samples of test material and the simultaneous amplification of DNA fragments of the detected microorganism and DNA of the human β-globin gene with hybridization-fluorescence detection. DNA of the β-globin gene is used as an endogenous internal control (IC Glob) and allows not only to control all stages of the PCR study for each sample, but also to evaluate the adequacy of the material and its storage.

Amplification of DNA fragments with the use of specific primers and Taq-polymerase enzyme are performed with the DNA samples obtained at the extraction stage. 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 EBV/HHV6 QT PCR kit uses “hot-start”, which greatly reduces the frequency of nonspecifically primed reactions. “Hot-start” is guaranteed by using chemically modified polymerase (TaqF). The 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 deoxyuridine triphosphate (dUTP).

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

Table 1

Channel for fluorophore	FAM	JOE	Cy5
DNA-target	IC Glob DNA	*EBV* DNA	*HHV*6 DNA
Target gene	β-globin gene	LMP-gene	DNA polymerase catalytic subunit

3 CONTENT

eSens EBV/HHV6 QT PCR kit (ES3240A) includes:

Reagent	Description	Volume, ml	Quantity
PCR-mix-1-FRT *EBV* / *HHV*6 / Glob	clear liquid from colorless to light lilac colour	0.6	2 tubes
PCR-mix-2-FRT	colorless clear liquid	0.3	2 tubes
Polymerase (TaqF)	colorless clear liquid	0.03	2 tubes
RNA-buffer	colorless clear liquid	0.6	1 tube
DNA calibrator KSG1	colorless clear liquid	0.2	1 tube
DNA calibrator KSG2	colorless clear liquid	0.2	1 tube
Negative Control (C-)*	colorless clear liquid	1.2	2 tubes
Positive Control DNA *EBV* / *HHV*6 and human DNA**	colorless clear liquid	0.1	2 tubes

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

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

eSens EBV/HHV6 QT PCR kit is intended for 110 reactions (including controls).

eSens EBV/HHV6 QT excel (version 1.0.) for data processing and result generation.

4 ADDITIONAL REQUIREMENTS

For pretreatment

- Reagent for pretreatment of whole or cord blood
- Disposable screwed or tightly closed 1.5-ml tubes

For DNA extraction and amplification

- DNA extraction kit.
- Sterile 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 tubes:
  a) thin-walled 0.2-ml PCR tubes with optical transparent domed or flat caps if a plate-type instrument is used;
  b) thin-walled 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.
- Pipettes (adjustable).
- Refrigerator for 2–8 °C.
- Deep-freezer at the temperature from minus 24 to minus 16 °C.
- Reservoir for used tips.
- Disposable powder-free gloves and a laboratory coat.

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 the PCR kit if the internal packaging was damaged or its appearance was changed.
- Do not use the PCR kit if the transportation and storage conditions according to the Instruction Manual were not observed.
- 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 a biological cabinet in compliance with appropriate biosafety practices.
- Clean and disinfect all samples or reagent spills using a disinfectant, such as 0.5 % sodium hypochlorite or other 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.
- While observing the conditions of transportation, operation and storage, there are no risks of explosion and ignition.
- Safety Data Sheets (SDS) are available on request.
- The PCR kit is intended for single use for PCR analysis of specified number of samples (see the section “Content”).
- The PCR kit is ready for use in accordance with the Instruction Manual. Use the PCR kit strictly for intended purpose.
- 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 EBV/HHV6 QT PCR kit is intended for analysis of the DNA extracted with DNA extraction kits from the clinical material (whole blood, white blood cells, viscera biopsy material and cerebrospinal fluid).

7 WORKING CONDITIONS

eSens EBV/HHV6 QT PCR kit should be used at the temperature from 20 to 28 °C and relative humidity from 15 to 75 %.

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)
  - **DNA-sorb-B** (K1-2-100-CE)

- For the automatic extraction
  - **ePure Viral Nucleic acid Extraction Kit** (E2003)

In the extraction procedure it is necessary to carry out the control reactions as follows:

C–	–	Add **100 µl of Negative Control (C–)** to the tube labelled C– (Negative Control of Extraction).
PCE	–	Add **90 µl of Negative Control (C–)** and **10 µl of Positive Control DNA *EBV* / *HHV6* and human DNA** to the tube labelled PCE (Positive Control of Extraction).

NOTE: Extract the DNA according to the manufacturer's protocol.

8.2 Preparing PCR

8.2.1 Preparing tubes for 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 DNA sample is **10 µl**.

1. Prepare the mixture of **PCR-mix-2-FRT** and **polymerase (TaqF)**. For this purpose transfer the content of the tube with **polymerase (TaqF) (30 µl)** into the tube with **PCR-mix-2-FRT (300 µl)** and mix by vortexing avoiding forming. Mark the tube by the date of mixture preparation.

NOTE: The prepared mixture is intended for analysis of 60 samples. The mixture is to be stored at 2–8 °C for 3 months. Use when needed.

NOTE: If the mixture cannot be used up for 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)**. The obtained mixture is intended for 30 reactions.

2. Prepare the reaction mixture.

NOTE: Even for analysis of **one** DNA sample in the **qualitative format**, it is necessary to run **two controls** of amplification: the Positive Control of Amplification (**KSG2**) and the Negative Control of Amplification (**RNA-buffer**). And even for analysis of **one** DNA sample in the **quantitative format**, it is necessary to run **five controls** of amplification: two calibrators (**KSG1** and **KSG2**) in two replicates and the Negative Control of Amplification (**RNA-buffer**). In addition, you should take reagents for one extra reaction.

3. Mix **PCR-mix-1-FRT *EBV* / *HHV6* / Glob** and the mixture of **PCR-mix-2-FRT** and **polymerase (TaqF)** prepared before in an individual tube in the following proportion:

    - **10 µl** of **PCR-mix-1-FRT *EBV* / *HHV6* / Glob**,
    - **5 µl** of mixture of **PCR-mix-2-FRT** and **polymerase (TaqF)**.

Calculate the required number of reaction including test and control samples, see Table 2.

Table 2

Scheme of reaction mixture preparation

Total reaction volume is 25 µl, volume of DNA sample is 10 µl			
Reagent volume for 1 reaction (µl)		**10.0**	**5.0**
Quantity of clinical samples		**PCR-mix-1-FRT *EBV* / *HHV*6 / Glob***	**mix of PCR-mix-2-FRT and polymerase (TaqF)***
For quantitative analysis	**For qualitative analysis**		
1	**4**	**70**	**35**
2	**5**	**80**	**40**
3	**6**	**90**	**45**
4	**7**	**100**	**50**
5	**8**	**110**	**55**
6	**9**	**120**	**60**
7	**10**	**130**	**65**
8	**11**	**140**	**70**
9	**12**	**150**	**75**
10	**13**	**160**	**80**
11	**14**	**170**	**85**
12	**15**	**180**	**90**
13	**16**	**190**	**95**
14	**17**	**200**	**100**
15	**18**	**210**	**105**
16	**19**	**220**	**110**
17	**20**	**230**	**115**
18	**21**	**240**	**120**
19	**22**	**250**	**125**
20	**23**	**260**	**130**
21	**24**	**270**	**135**
22	**25**	**280**	**140**
23	**26**	**290**	**145**
24	**27**	**300**	**150**
25	**28**	**310**	**155**
30	**33**	**360**	**180**

***** Values are given with account of one extra reaction and five controls (2 DNA calibrators KSG1 and KSG2 (in two replicates), negative control (RNA-buffer) for quantitative analysis of DNA, and two controls (positive and negative) for qualitative analysis of DNA.

4. Take the required number of tubes for amplification of test and control DNA samples. Transfer **15 µl** of the prepared mixture into each tube. Add **10 µl** of **DNA** obtained at the DNA extraction stage to the tubes with the reaction mixture.
5. Carry out the control reactions:

For qualitative analysis:

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

C+ – Add **10 µl** of **DNA calibrator KSG2** to the tube labeled C+ (Positive Control of Amplification).

C– – Add **10 µl** of **the sample extracted from the Negative Control reagent** to the tube labeled C– (Negative control of Extraction).

PCE – Add **10 µl** of **the sample extracted from the Positive Control DNA *EBV* / *HHV6* and human DNA reagent** to the tube labeled PCE (Positive control of Extraction).

For quantitative analysis:

NCA	–	Add **10 µl** of **RNA-buffer** to the tube labeled NCA (Negative Control of Amplification).
Calibrators KSG1 and KSG2	–	Add **10 µl** of **KSG1** to two tubes and **10 µl** of **KSG2** to other two tubes.
C–	–	Add **10 µl** of **the sample extracted from the Negative Control reagent** to the tube labeled C– (Negative control of Extraction).
PCE	–	Add **10 µl** of **the sample extracted from the Positive Control DNA *EBV* / *HHV6* and human DNA reagent to the tube labeled PCE (Positive control of Extraction).**

8.2.2 Amplification

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

Table 3

eSens-1 amplification 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**	**Cycles**	**Temperature, °C**	**Time**	**Cycles**
1	**95**	**15 min**	**1**	**95**	**15 min**	**1**
2	**95**	**5 s**	**5**	**95**	**5 s**	**5**
	60	**20 s**		**60**	**20 s**	
	72	**15 s**		**72**	**15 s**	
3	**95**	**5 s**	**40**	**95**	**5 s**	**40**
	60	**20 s**		**60**	**30 s**	
	72	**15 s**		**72**	**15 s**	

Fluorescent signal is detected in the channels for the **FAM, JOE** and **Cy5** fluorophores.

2. Adjust the fluorescence channel sensitivity according to the *Technical Sheet*.
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

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

- The signal of the β-Globin gene DNA (IC Glob) amplification product is detected in the channel for the FAM fluorophore.
- The signal of the *EBV* DNA amplification product is detected in the channel for the JOE fluorophore.
- The signal of the *HHV*6 DNA is detected in the channel for the Cy5 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 DNA sample in the corresponding column of the results grid.

Principle of interpretation is the following:

- ***EBV* DNA** is **detected** 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 *Technical Sheet.* Moreover, the fluorescence curve of the sample should cross the threshold line in the area of typical exponential growth of fluorescence.
- ***HHV*6 DNA** is **detected** if the *Ct* value determined in the results grid in the channel for the **Cy5** fluorophore does not exceed the boundary *Ct* value specified in the *Technical Sheet.* Moreover,

the fluorescence curve of the sample should cross the threshold line in the area of typical exponential growth of fluorescence.

- ***EBV* DNA is not detected** if the *Ct* value is not determined (absent) in the results grid in the channel for the JOE fluorophore (the fluorescence curve does not cross the threshold line) and ***HHV*6 DNA is not detected** if the *Ct* value is not determined (absent) in the results grid in the channel for the Cy5 fluorophore (the fluorescence curve does not cross the threshold line). Whereas for qualitative analysis the *Ct* value in the results grid in the channel for the FAM fluorophore should not exceed the *Ct* value specified in the *Technical Sheet,* and for quantitative analysis, the quantity of IC Glob DNA should be more than 2000 copies/reaction for whole blood, white blood cells, viscera biopsy material.

NOTE: For cerebrospinal fluid, the *Ct* value could be greater than the *Ct* value in the channel for FAM fluorophore specified in the *Technical Sheet* or the quantity of IC Glob DNA could be less than 500 copies/reaction in case of quantitative analysis because the cerebrospinal fluid samples may contain a very small number of cells.

- The result of analysis is **invalid** if the *Ct* value is not determined (absent) in the results grid or greater than the boundary *Ct* value in the channels for the JOE or Cy5 fluorophores. Whereas the *Ct* value in the results grid in the channel for the FAM fluorophore is greater than the *Ct* value specified in the *Technical Sheet* (for qualitative analysis) or the quantity of IC Glob DNA is less than 2000 copies/reaction for whole blood, white blood cells, viscera biopsy material (for quantitative analysis). In such case the PCR analysis should be repeated for required sample.
- The result is **equivocal** for the clinical samples with the *Ct* value determined in the channels for the JOE or Cy5 fluorophores greater than the boundary *Ct* value specified in the *Technical Sheet*. In that case, it is necessary to conduct additional analysis for that DNA sample with two repeats. If the repeated positive *Ct* value is obtained, the result is considered positive. If the positive *Ct* value can't be reproduced in two repeats, the result is considered **equivocal.**
- The negative result is considered **unreliable** if the *Ct* value in the channel for the FAM fluorophore is greater than the boundary *Ct* value specified in the *Technical Sheet* (for qualitative analysis). The positive or negative results (the quantitative analysis) are considered **unreliable** if the quantity of IC Glob DNA is less than 2000 copies/reaction for whole blood, white blood cells, viscera biopsy material.

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 and enclosed *Technical Sheet*). For quantitative analysis the results for Positive Control should fall in the concentration range specified in the *Technical Sheet*.

Table 4

Results for controls in qualitative analysis

Control	Stage for control	*Ct* in the channel for fluorophore		
		FAM	JOE	Cy5
C–	DNA extraction, PCR	Absent	Absent	Absent
PCE	DNA extraction, PCR	<boundary value	<boundary value	<boundary value
NCA	PCR	Absent	Absent	Absent
C+ (for qualitative analysis)	PCR	<boundary value	<boundary value	<boundary value

Table 5

Results for controls in quantitative analysis

Control	Stage for control	*Ct* in the channel for fluorophore		
		FAM	JOE	Cy5
C–	DNA extraction, PCR	Absent	Absent	Absent
PCE	DNA extraction, PCR	<boundary value	concentration value falls in the range specified in the *Technical Sheet*	concentration value falls in the range specified in the *Technical Sheet*
NCA	PCR	Absent	Absent	Absent
KSG1, KSG2	PCR	*Ct* value and calculated concentration are defined	*Ct* value and calculated concentration are defined	*Ct* value and calculated concentration are defined

For quantitative analysis, if total DNA is extracted from human whole blood, white blood cells, and viscera biopsy material, the concentration in log of DNA copies per standard cell quantity (10^5) in control and test samples is calculated according to the following formula:

For *EBV*:

$$\lg\left\{\frac{\text{number of } EBV \text{ DNA copies in PCR sample}}{\text{number of Glob DNA copies in PCR sample}} \text{ x 2 x } 10^5\right\} = \lg\{EBV \text{ DNA copies}/10^5 \text{ cells}\}$$

For *HHV6*:

$$\lg\left\{\frac{\text{number of } HHV6 \text{ DNA copies in PCR sample}}{\text{number of Glob DNA copies in PCR sample}} \text{ x 2 x } 10^5\right\} = \lg\{HHV6 \text{ DNA copies}/10^5 \text{ cells}\}$$

If total DNA is extracted from cerebrospinal fluid (liquor), the concentration of DNA per ml of clinical sample (CS $_{DNA}$) is calculated according to the following formula:

CS $_{DNA}$ = number of DNA copies *EBV*, *HHV*6 in PCR sample x 100 (copies/ml)

10 TROUBLESHOOTING

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

1. If any *Ct* value appears in the channels for the FAM, JOE and Cy5 fluorophores for the Negative Control of Amplification (NCA) and Negative Control of Extraction (C–) these results testify the presence of contamination of reagents or samples. In that case the PCR-analysis should be repeated (beginning with the extraction stage) for all samples, in which DNA was detected.
2. If the *Ct* value is absent or greater than the boundary value in the results grid for the Positive Control of Amplification (C+) – **KSG2** – for the qualitative analysis in the channels for the JOE, FAM or Cy5 fluorophores, the amplification must be repeated for all samples where pathogen agent DNA was not detected.
3. If the *Ct* value is absent or greater than the boundary value for the Positive Control of Extraction (PCE) – **Positive Control DNA *EBV* / *HHV*6 and human DNA –** in the channels for the JOE, FAM, or Cy5 fluorophores, the results of analysis must be considered as **invalid** for all samples. PCR should be repeated for all samples.
4. If the *Ct* value for given sample was not defined or the *Ct* value exceeds the boundary value in the channel for the JOE, or Cy5 fluorophores, and *Ct* value defined in the channel for the FAM fluorophore exceeds the maximal value specified for IC, the experiment needs to be repeated, starting with the extraction stage. Possible reason is an error in the clinical material pretreatment procedure that leads to the DNA loss or the presence of PCR inhibitors.
5. If the *Ct* value for the clinical samples exceeds the maximal boundary value in the channel for the JOE or Cy5 fluorophore, the results of analysis must be considered as **equivocal**. In that case, it is necessary to conduct additional analysis for that DNA sample with two repeats. If the repeated positive *Ct* value is obtained, the result is considered positive. If the positive *Ct* value can't be reproduced in two repeats, the result is considered **equivocal**.
6. If in quantitative analysis the copies/reaction values in calibrators differ by more than 30 % from the set values, it is necessary to check the tube order in the rotor (calibrators should be placed in the wells indicated as ***Standard*** in sample table, concentration should correspond to concentration specified in the *Technical Sheet*, well no.1 must be filled with some test tube (not empty)).
7. If the correlation coefficient R in ***Standard Curve*** window is less than 0.9 (in case of quantitative analysis), it means that calibration failed. Check the settings of calibrators and correct inaccuracies, if no effect, repeat PCR for all samples and calibrators.

11 TRANSPORTATION

eSens EBV/HHV6 QT PCR kit should be transported at 2–8 °C for no longer than 5 days.

12 STABILITY AND STORAGE

All components of the **eSens EBV/HHV6 QT PCR kit** are to be stored at 2–8 °C when not in use (except for PCR-mix-1-FRT *EBV* / *HHV*6 / Glob, PCR-mix-2-FRT and polymerase (TaqF)). All components of the **eSens EBV/HHV6 QT 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: PCR-mix-1-FRT *EBV* / *HHV6* / Glob, PCR-mix-2-FRT, and polymerase (TaqF) are to be stored at the temperature from minus 24 to minus 16 °C.

NOTE: PCR-mix-1-FRT *EBV* / *HHV6* / Glob is to be kept away from light.

13 SPECIFICATIONS

13.1 Analytical sensitivity

Clinical material	Nucleic acid extraction kit	Analytical sensitivity
Cerebrospinal fluid (liquor)	RIBO-prep ePure Viral Nucleic acid extraction kit	400 copies/ml
Whole blood, white blood cells, viscera biopsy material	RIBO-prep ePure Viral Nucleic acid extraction kit	5 DNA copies per 10^5 cells

13.2 Analytical specificity

eSens EBV/HHV6 QT PCR kit is intended for *Epstein-Barr virus* (*EBV*) DNA and *Human Herpes Virus* type 6 (*HHV*6) DNA. Specific activity of **eSens EBV/HHV6 QT PCR kit** was confirmed by analysis of QCMD panel for *Epstein-Barr virus,* as well as by analysis of clinical material with subsequent confirmation of the results by sequencing the amplified fragments.
The activity of the PCR kit components with respect to DNA of other viruses (herpes simplex virus types 1 and 2, human herpes virus type 8, Varicella Zoster Virus, Parvovirus B19, and others), bacterial pathogens (*Staphylococcus aureus*, *Streptococcus pyogenes*, *Streptococcus agalactiae*, and others) and human DNA was absent.
The clinical specificity of **eSens EBV/HHV6 QT 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
LOT	Batch code
IVD	*In vitro* diagnostic medical device
VER	Version
	Temperature limit
	Manufacturer
	Date of manufacture
EC REP	Authorized representative in the European Community
	Caution
	Contains sufficient for <n> tests
	Use-by Date
	Consult instructions for use
	Keep away from sunlight
NCA	Negative control of amplification
C-	Negative control of extraction
C+	Positive control of amplification
PCE	Positive Control of Extraction

List of Changes Made in the Instruction Manual

VER	Location of changes	Essence of changes
01_04/2022		

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