ECOLI

eSens

CE

IVD

For Professional Use Only

eSens Florocenosis/Candida QT PCR kit

REF ES3046A

Instructions for Use

1 INTENDED USE

eSens Florocenosis/Candida QT PCR kit is an *in vitro* nucleic acid amplification test for simultaneous detection and quantitation of *Candida* genus fungi DNA (*C.albicans, C.glabrata, C.krusei, C.parapsilosis* and *C.tropicalis*) in the clinical material (urogenital swabs, oral and oropharyngeal swabs and urine samples) by using polymerase chain reaction with real-time hybridization-fluorescence detection products of amplification.

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

2 PRINCIPLE OF PCR DETECTION

Qualitative detection and quantitation of five types of *Candida* spp. DNA by the multiplex polymerase chain reaction (PCR) with real-time hybridization-fluorescence detection contains three steps: DNA extraction from the clinical material, amplification of the given microorganism DNA and real-time hybridization-fluorescence detection.

Candida spp. detection by the polymerase chain reaction (PCR) is based on the amplification of the pathogen genome specific region using specific 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 Florocenosis/Candida QT PCR kit is a test that contains the Internal Control (Internal Control-FL (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.

The results of amplification of *C.albicans, C.glabrata* and *C.krusei* DNA are registered separately for each species in three different channels. The results of amplification of *C.parapsilosis* and *C.tropicalis* DNA are registered together in the fourth channel. The Internal Control-FL (IC) amplification product is detected in the Cy5.5/Crimson channel.

Detection channel	FAM*	JOE*	ROX*	Cy5*	Cy5.5*
Identified DNA	DNA *C.albicans*	DNA *C.glabrata*	DNA *C.krusei*	DNA *C.parapsilosis* and *C.tropicalis*	DNA IC

* Or the similar detection channel for the detection of the specified fluorophore in accordance with the using instrument.

The quantitation of DNA by real-time PCR is based on the existence of linear dependence between the logarithm of initial DNA-target concentration in the sample and the threshold cycle *Ct*, which corresponds to the start of the exponential growth of the fluorescent signal. The simultaneous amplification with real-time detection of DNA samples and DNA calibrators with the known concentrations is carried out for qualitative analysis. Calibration curve is plotted automatically on the basis of DNA calibrator results. Concentration of corresponding DNA-target is calculated automatically for each sample using the obtained value of threshold cycle and the calibration curve.

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.

3 CONTENT

eSens Florocenosis/Candida QT PCR kit (ES3046A) includes:

Reagent		Description	Volume, ml	Quantity
PCR-mix-1-FL Florocenosis / *Candida*		clear liquid from colorless to blue grey colour	1.2	1 tube
PCR-mix-2-FRT		colorless clear liquid	0.3	2 tubes
Polymerase (TaqF)		colorless clear liquid	0.03	2 tubes
DNA-buffer		colorless clear liquid	0.5	1 tube
DNA calibrators	**CND1**	colorless clear liquid	0.2	1 tube
	CND2	colorless clear liquid	0.2	1 tube
Negative control (C–)*		colorless clear liquid	1.2	1 tube
Internal Control-FL (IC)**		colorless clear liquid	1.0	1 tube

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

** add 10 µl of Internal Control-FL (IC) during the DNA extraction procedure directly to the sample/lysis mixture.

eSens Florocenosis/Candida QT PCR kit is intended for 110 reactions (including controls).

4 ADDITIONAL REQUIREMENTS

- Transport medium for storage and transportation of swabs.
- DNA extraction kit.
- PCR box.
- Vortex mixer.
- Desktop centrifuge with a rotor for 2-ml reaction tubes.
- Disposable pipette tips with filter (up to 100 µl) in racks (for example, Axygen, USA).
- Pipettes (adjustable).
- Disposable powder-free gloves and laboratory coat.
- Tube racks.
- Real-time instruments with five or more separated channels of fluorescence detection (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 with the range from 2 to 8 °C.
- Deep-freezer with the 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 Florocenosis/Candida QT PCR kit is used for simultaneous detection and quantitation of *Candida* genus fungi DNA (*C.albicans, C.glabrata, C.krusei, C.parapsilosis* and *C.tropicalis*) extracted with DNA extraction kit from the clinical material (urogenital swabs, oral and oropharyngeal swabs, transferred into the **Transport Medium with Mucolytic Agent** and urine samples).

7 WORKING CONDITIONS

eSens Florocenosis/Candida QT 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.

It is recommended to use the following nucleic acid extraction kits:

– **DNA-sorb-AM**.

For the automatic extraction

– **ePure STD DNA Extraction Kit (E2007)**

Extract the DNA according to the manufacturer's protocol.

NOTE: It is forbidden to use EDEM reagents kit or other express methods for DNA extraction.

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. Previously prepare the mixture of **PCR-mix-2-FRT** and **polymerase (TaqF)**. The content of one tube with **polymerase (TaqF) (30 µl)** should be transferred into the tube with the **PCR-mix-2–FRT (300 µl)**. Mix carefully avoiding foaming. Mark the tubes by the expiration date.

NOTE: The prepared mixture intended for 60 reactions. The mixture should be stored at 2–8 °C for 3 months and used when it is necessary.

If the mixture is not to be used in three months, the mixture should be prepared for less number of reactions. For example, mix 150 µl of PCR-mix-2-FRT and 15 µl of polymerase (TaqF) for 30 reactions.

2. Vortex the tube with PCR-mix-1-FL Florocenosis / Candida, sediment the drops from the tubes cap by short centrifugation.

   Calculate the reagents volumes for the necessary number of reactions including test and control samples analysis according to the table 1. Take into account that even for one test sample analysis four control reactions are to be carried out CND1, CND2, NCA and C–.

   The reagents should be taken with reserve. For N samples analysis the reagents for (N+1) reactions should be prepared.

Table 1

Scheme of reaction mixture preparation

	Reagent volume for the specified number of reactions, µl	
Reagent volume per one reaction, µl	10.0	5.0
Number of examining clinical samples	PCR-mix-1-FL Florocenosis / *Candida**	Mixture of PCR-mix-2-FRT and polymerase (TaqF)*
1	60	30
2	70	35
3	80	40
4	90	45
5	100	50
6	110	55
7	120	60
8	130	65
9	140	70
10	150	75
11	160	80
12	170	85
13	180	90
14	190	95
15	200	100
16	210	105
17	220	110
18	230	115
19	240	120

20	250	125
21	260	130
22	270	135
23	280	140
24	290	145
25	300	150
30	350	175

* Specified values include one extra reaction and four controls (CND1, CND2, NCA and C–).

3. Prepare the reaction mixture in another tube. The components of the reaction mixture should be mixed directly before the experiment. For one reaction mix:
    - 10 µl of PCR-mix-1-FL Florocenosis / *Candida*
    - 5 µl of PCR-mix-2–FRT and polymerase (TaqF) mixture.
4. Take the required number of tubes/strips for amplification of the DNA obtained from clinical and control samples.
5. Add **15 µl** of reaction mixture into each tube.
6. Using tips with aerosol filter, add **10 µl** of **DNA samples** obtained at the DNA extraction stage.
7. Carry out the control reactions:

C–	—	Add **10 µl** of the sample extracted from the **Negative Control** (C–) reagent to the tube labeled C– (Negative Control of Extraction).
NCA	—	Add **10 µl** of **DNA-buffer** to the tube labeled NCA (Negative control of amplification).
CND1, CND2	—	Add **10 µl** of DNA calibrator **CND1** into one tube and **10 µl** of DNA calibrator **CND2** into another tube.

8.2.2 Amplification

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

Table 2

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 Fluorescent signal detection		60	30 s Fluorescent signal detection	
	72	15 s		72	15 s	

Fluorescent signal is detected in the channels for the FAM, JOE, ROX, Cy5 and Cy5.5 fluorophores.

2. Insert tubes into the reaction module of the device.
3. Run the amplification program with fluorescence detection.
4. Analyze results after the amplification program is completed.

9 DATA ANALYSIS

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

- The signal of the ***C.albicans*** DNA amplification product is detected in the channel for the **FAM** fluorophore.
- The signal of the ***C.glabrata*** DNA amplification product is detected in the channel for the **JOE** fluorophore.
- The signal of the ***C.krusei*** DNA amplification product is detected in the channel for the **ROX** fluorophore.
- The signal of the ***C.parapsilosis*** and/or ***C.tropicalis*** DNA amplification product is detected in the channel for the **Cy5** fluorophore.
- The signal of the **Internal Control (IC)** DNA amplification product is detected through the channel for the **Cy5.5** fluorophore.

Results are interpreted by the crossing (or not-crossing) the fluorescence curve with the threshold line set at the level of exponential growth that corresponds the presence (or absence) of a *Ct* value for the certain DNA-target in the corresponding column of the result grid. The calibration curve is plotted and the concentration of detected species of *Candida* spp. is calculated automatically according to the *Ct* values of the DNA calibrators.

NOTE: The concentrations values of DNA calibrators are specified in the *Technical sheet* enclosed in the PCR kit.

The result of analysis is considered reliable only if the results obtained for Negative Control of amplification as well as for the Negative Control of extraction of DNA and DNA calibrator CND2 are correct (see Table 3) and the amplification efficiency coefficient E is in the range specified in the *Technical sheet* enclosed in the PCR kit.

Table 3

Results for controls of different stages of the PCR

Control	**Stage for control**	***Ct* value in the channel for fluorophore**	
		FAM, JOE, ROX, Cy5	**Cy5.5**
C–	DNA extraction	Absent	<boundary value
NCA	PCR	Absent	Absent
CND2	PCR	<boundary value	Not estimated

The concentration values of *C.albicans, C.glabrata, C.krusei, C.parapsilosis* and *C.tropicalis* DNA reflect the total content of those microorganisms in the clinical material transferred into the transport medium. The initial values of number of copies of *C.albicans, C.glabrata, C.krusei* and *C.parapsilosis+C.tropicalis* DNA in the reaction tube are automatically calculated and given in the corresponding column of the results grid (see *Technical sheet*) on the basis of the set values of DNA calibrators. The obtained values are used for the calculation of number of genome equivalents of the corresponding species of *Candida* in 1 ml of the initial clinical material using the formula:

[Number of copies] *Candida* DNA x K = [Number of genome equivalents] in 1 ml (GE/ml)

NOTE: The coefficient K for calculations in GE/ml is specified in the *Technical sheet* enclosed in the PCR kit.

If the obtained result is greater than $2x10^5$ GE/ml then the result “greater than $2x10^5$ GE/ml” is specified, if the obtained result is less than 200 GE/ml then the result “less than 200 GE/ml” is specified (taking into account the linear range of the kit).

The clinical interpretation of the test results should be carried out by the doctor only on the basis of complex examination of the patient according to the anamnesis data, clinical and epidemiological status, keeping into account the existed clinical and methodological recommendations.

10 TROUBLESHOOTING

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

1. If the *Ct* value is determined for the Negative Control of extraction (C–) and/or Negative Control of amplification (NCA) in the channels for the FAM and/or JOE, and/or ROX, and/or Cy5 fluorophores, the PCR should be repeated for all the samples for which the *Ct* value is defined in the channels for the FAM and/or JOE, and/or ROX, and/or Cy5 fluorophores.
2. If the *Ct* value determined for the DNA calibrators (CND1, CND2) in the channels for the FAM, JOE, ROX, Cy5 fluorophores is greater than the boundary value or absent, or the efficiency coefficient E on the standards curve is less than the value specified in the *Technical sheet,* the amplification should be repeated for all of the samples.

3. If the *Ct* values for the test sample in the channels for the FAM, JOE, ROX, Cy5 fluorophores are absent or the obtained number of DNA copies is less than 100 and the *Ct* value in the channel for the Cy5.5 fluorophore is greater than the boundary value or absent, the analysis should be repeated for this sample starting from the stage of DNA extraction.

11 TRANSPORTATION

eSens Florocenosis/Candida 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 Florocenosis/Candida QT PCR kit** are to be stored at 2–8 °C when not in use (except for PCR-mix-2-FRT and polymerase (TaqF)).

All components of the **eSens Florocenosis/Candida QT PCR kit** are stable until the expiry date stated on the label. **eSens Florocenosis/Candida QT PCR kit** can be stored without unpacking at 2 to 8 °C for 3 months from the date of manufacture before opening. Once opened, **eSens Florocenosis/Candida QT PCR kit** should be stored in accordance with the storage temperatures for each component. The shelf life of reagents before and after the first use is the same, unless otherwise stated.

NOTE: 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-FL Florocenosis / Candida is to be kept away from light.

13 SPECIFICATIONS

13.1 Analytical sensitivity

Clinical material	**Transport medium**	**Nucleic acid extraction kit**	**Micro-organism**	**PCR kit**	**Sensitivity, GE/ml***
Urogenital swabs, oral and oropharyngeal swabs	Transport Medium for Swabs or Transport Medium with Mucolytic Agent	DNA-sorb-AM ePure STD DNA Extraction Kit	*C.albicans*, *C.glabrata*, *C.krusei*, *C.parapsilosis*, *C.tropicalis*	eSens Florocenosis/ Candida QT PCR kit	$1x10^2$
Urine**	—	DNA-sorb-AM ePure STD DNA Extraction Kit	*C.albicans*, *C.glabrata*, *C.krusei*, *C.parapsilosis*, *C.tropicalis*	eSens Florocenosis/ Candida QT PCR kit	$1x10^2$

* Genome equivalents (GE) of the pathogen agent per 1 ml of a sample.
** For urine samples the pretreatment for sediment obtaining from 1 ml of urine is required.

13.2 Analytical specificity

The analytical specificity of **eSens Florocenosis/Candida QT PCR kit** is ensured by the selection of specific primers and probes as well as stringent reaction conditions. The primers and probes have been checked for possible homologies to all sequences published in gene banks by sequence comparison analysis.

The nonspecific reactions were absent when testing the human DNA samples and the following microorganisms' DNA: *Candida albicans, C.glabrata, C.krusei, C.parapsilosis, C.tropicalis, Gardnerella vaginalis, Lactobacillus* spp., *Escherichia coli, Staphylococcus* spp., *Streptococcus* spp., *Chlamydia trachomatis, Neisseria gonorrhoeae, Neisseria* spp., *Mycoplasma genitalium, Trichomonas vaginalis, Treponema pallidum, Toxoplasma gondii, HSV* 1 and 2 types, *CMV, HPV*.

The clinical specificity of **eSens Florocenosis/Candida QT PCR kit** was confirmed in laboratory clinical trials.

13.3 Linear range

The linear range of measurements for quantitative detection of each detected microorganisms is from 200 to $2x10^5$ GE/ml.

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		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	IC	Internal control

List of Changes Made in the Instruction Manual

VER	Location of changes	Essence of changes
01_04/2022		

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