ECOLI

eSens

CE

IVD

For Professional Use Only

eSens Candida albicans/glabrata/krusei QL PCR kit

REF ES3041A

Instructions for Use

1 INTENDED USE

eSens Candida albicans/glabrata/krusei QL PCR kit is an *in vitro* nucleic acid amplification test for simultaneous detection of DNA of *Candida albicans, Candida glabrata,* and *Candida krusei* in the clinical material (urogenital, rectal, and pharyngeal swabs; conjunctival discharge; prostate gland secretion; and urine samples) 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

C.albicans / C.glabrata / C.krusei DNA detection by the polymerase chain reaction (PCR) is based on the amplification of the pathogen genome specific region using specific *C.albicans / C.glabrata / C.krusei* primers. In 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 Candida albicans/glabrata/krusei QL PCR kit is a qualitative 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.

eSens Candida albicans/glabrata/krusei 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). 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 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	ROX	Cy5
DNA-target	*Candida albicans* DNA	*Candida glabrata* DNA	*Candida krusei* DNA	Internal Control
Target gene	gene ITS-2	gene ITS-2	gene ITS-2	Artificially synthesized sequence

3 CONTENT

eSens Candida albicans/glabrata/krusei QL PCR kit (ES3041A) contains:

Reagent	Description	Volume, ml	Quantity
PCR-mix-1-FL *C.albicans / C.glabrata / C.krusei*	clear liquid from colorless to light lilac 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
Positive Control complex (C+)	colorless clear liquid	0.2	1 tube
DNA-buffer	colorless clear liquid	0.5	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 Candida albicans/glabrata/krusei QL PCR kit is intended for 110 reactions (including controls).

4 ADDITIONAL REQUIREMENTS

- DNA extraction kit.
- Transport medium.
- Disposable powder-free gloves and laboratory coat.
- Pipettes (adjustable).

- 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 :
- thin-walled 0.2-ml PCR tubes with optical transparent domed or flat caps or strips of eight 0.2-ml tubes with optical transparent caps if a plate-type instrument is used;
- 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.
- Refrigerator for 2–8 °C.
- Deep-freezer 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 Candida albicans/glabrata/krusei QL PCR kit is intended for the analysis of DNA extracted with DNA extraction kits from the clinical material (urogenital, rectal, and pharyngeal swabs; conjunctival

discharge; prostate gland secretion; urine samples (sediment of the first portion of the morning specimen)).

7 WORKING CONDITIONS

eSens Candida albicans/glabrata/krusei QL PCR kit should be used at 18–25 °C.

8 PROTOCOL

8.1 DNA extraction

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

Ecoli Dx, s.r.o. recommends:

- For the manual extraction

- **DNA-sorb-AM** (K1-12-100-CE)

- For the automatic extraction

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

The DNA extraction for each sample is carried out in the presence of **Internal Control-FL (IC).**

Please carry out nucleic acid extraction according to the manufacture´s instruction.

8.2 Preparing 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.

8.2.1 Preparing tubes for PCR

The total reaction volume is **25 µl**, the volume of DNA sample is **10 µl**.

1. Thaw the required number of tubes with **PCR-mix-2-FRT**. Vortex the tubes with **PCR-mix-1-FL *C.albicans / C.glabrata / C.krusei*, PCR-mix-2-FRT, and polymerase (TaqF)** and sediment the drops by short centrifugation (1-2 s).

   Take the required number of tubes/strips for amplification of the cDNA obtained from clinical and control samples.

2. For N reactions (including 2 controls), add to a new tube:

- **10*(N+1) µl of PCR-mix-1-FL C.albicans / C.glabrata / C.krusei,**
- **5.0*(N+1) µl of PCR-mix-2-FRT,**
- **0.5*(N+1) µl of polymerase (TaqF).**

   Mix the prepared mixture and sediment the drops by short centrifugation (1-2 s). Transfer **15 µl** of the prepared mixture to prepared tubes.
3. Add **10 µl** of **DNA** obtained at the DNA extraction stage to the prepared tubes.
4. Carry out the control amplification reactions:

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

C+	Add 10 µl of Positive Control complex (C+) to the tube labeled C+ (Positive Control of Amplification).
C–	Add 10 µl of sample extracted from the Negative Control (C–) reagent to the tube labeled C– (Negative Control of Extraction).

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 Fluorescence acquiring		60	30 s Fluorescence acquiring	
	72	15 s		72	15 s	

Fluorescent signal is detected in the channels for the FAM, JOE, ROX, and Cy5 fluorophores. Other channels are enabled if several tests are simultaneously carried out in a single run.

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 instruments

Channel	**Calibrate/Gain Optimisation…**	**Threshold**	**Dynamic tube**	**Slope Correct**	**More Settings/ Outlier Removal**
FAM/Green	from 5 FI to 10 FI	0.1	On	Off	0%

JOE/Yellow	from 4 FI to 8 FI	0.1	On	Off	5%
ROX/Orange	from 4 FI to 8 FI	0.1	On	Off	5%
Cy5/Red	from 4 FI to 8 FI	0.07	On	On	5-10 %

Test settings for plate-type instruments

Set the heating/cooling ***Ramp Rate* 2,5 °C/s**.

Channel	**Threshold**
FAM, HEX, ROX, Cy5	For each channel in Log Scale set the threshold line al the level of 10-20 % of maximum fluorescence obtained for the Positive Control of Amplification (C+) in the last amplification cycle

9 DATA ANALYSIS

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

- The signal of the *Candida albicans* DNA amplification product is detected in the channel for the FAM fluorophore.
- The signal of the *Candida glabrata* DNA amplification product is detected in the channel for the JOE fluorophore.
- The signal of the *Candida krusei* DNA amplification product is detected in the channel for the ROX fluorophore
- The signal of the (IC) DNA amplification product 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:

- *Candida albicans* DNA is **detected** in a sample if the *Ct* value is determined in the results grid in the channel for the FAM fluorophore. Moreover, the fluorescence curve of the sample should cross the threshold line in the area of typical exponential growth of fluorescence.
- *Candida glabrata* DNA is **detected** in a sample if the *Ct* value is determined in the results grid in the channel for the JOE fluorophore. Moreover, the fluorescence curve of the sample should cross the threshold line in the area of typical exponential growth of fluorescence.
- *Candida krusei* DNA is **detected** in a sample if the *Ct* value is determined in the results grid in the channel for the ROX fluorophore. Moreover, the fluorescence curve of the sample should cross the threshold line in the area of typical exponential growth of fluorescence.
- *Candida albicans, Candida glabrata* and *Candida krusei* DNA are **not detected** if the *Ct* value is not determined (absent) in the results grid (the fluorescence curve does not cross the threshold line) in the channels for the FAM, JOE, ROX fluorophores, whereas the *Ct* value determined in the results grid in the channel for the Cy5 fluorophore does not exceed the specified boundary value.

The result of analysis is **invalid** if the *Ct* value is not determined in the results grid (absent) in the channel for the FAM, JOE, ROX and Cy5 fluorophore. In such cases PCR should be repeated for this sample.

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 Negative Control of extraction are correct (see Table 3 and 4).

Table 3

Results for controls

Control	**Stage for control**	***Ct* value in the channel for fluorophore**			
		FAM	**JOE**	**ROX**	**Cy5**
C–	DNA extraction	Absent	Absent	Absent	<boundary value
NCA	PCR	Absent	Absent	Absent	Absent
C+	PCR	<boundary value	<boundary value	<boundary value	<boundary value

Table 4

Boundary *Ct* values

	Rotor-type instrument				**Plate-type instrument**			
Sample	**Channel for fluorophore**							
	FAM	**JOE**	**ROX**	**Cy5**	**FAM**	**JOE**	**ROX**	**Cy5**
C+	<33	<33	<33	<33	<36	<36	<36	<36
C-	Ct is absent			<33	Ct is absent			<36
NCA	Ct is absent				Ct is absent			
Test samples	-	-	-	<33	-	-	-	<36

10 TROUBLESHOOTING

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

1. The *Ct* value determined for the Positive Control of amplification (C+) in the channel for the FAM**,** and/or JOE, and/or ROX fluorophore is greater than the specified boundary value or absent. The amplification should be repeated for all the samples in which *Ct* value is absent in the respective channel.
2. The *Ct* value is determined for the Negative Control of Extraction (C–) and/or the Negative Control of Amplification (NCA) in the channel for the FAM, and/or JOE, and/or ROX fluorophore. The PCR analysis (beginning with the DNA extraction stage) should be repeated for all samples in which *Ct* value is determined in the respective channel.

11 TRANSPORTATION

eSens Candida albicans/glabrata/krusei 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 Candida albicans/glabrata/krusei QL PCR kit** (except for polymerase (TaqF) and PCR-mix-2-FRT) are to be stored at 2–8 °C when not in use. All components of the **eSens Candida albicans/glabrata/krusei QL PCR kit** are stable until the expiry date on the label. PCR kit can be stored without unpacking at 2 to 8 °C for 3 months from the date of manufacture before opening. Once opened, PCR kit should be unpacked in accordance with the storage temperatures for each component. The shelf life of opened reagents is the same as that of unopened reagents, unless otherwise stated.

NOTE:

- Polymerase (TaqF) and PCR-mix-2-FRT are to be stored at temperature from minus 24 to minus 16 °C when not in use.
- PCR-mix-1-FL *C.albicans / C.glabrata / C.krusei* is to be kept away from light.

13 SPECIFICATIONS

13.1 Sensitivity

Clinical material	Transport medium	Nucleic acid extraction kit	Microorganism	Sensitivity, GE/ml*
Urogenital swabs	Transport Medium for Swabs or Transport Medium with Mucolytic Agent	DNA-sorb-AM ePure STD DNA Extraction Kit	*Candida albicans*	$1x10^3$
			Candida glabrata	$1x10^3$
			Candida krusei	$1x10^3$
Urine	—	DNA-sorb-AM ePure STD DNA Extraction Kit	*Candida albicans*	$2x10^3$
			Candida glabrata	$2x10^3$
			Candida krusei	$2x10^3$

*Genome equivalents of microorganism per 1 ml of the sample from transport medium.

NOTE: The analytical sensitivity of each microorganism does not change even at high concentrations of two other microorganisms (to 10^9 GE/ml).

13.2 Specificity

The analytical specificity of **eSens Candida albicans/glabrata/krusei QL PCR kit** is ensured by 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.

Nonspecific responses were absent while testing human DNA samples and DNA samples of the following microorganisms: *Gardnerella vaginalis, Lactobacillus* spp., *Escherichia coli, Staphylococcus* spp., *Streptococcus* spp., *Chlamydia trachomatis, Mycoplasma hominis, Ureaplasma urealyticum,*

Ureaplasma parvum, Mycoplasma genitalium, Candida albicans, Candida glabrata, Candida krusei, Neisseria spp., *Neisseria gonorrhoeae, Trichomonas vaginalis, Treponema pallidum, Toxoplasma gondii, HSV* types 1 and 2, *CMV,* and *HPV*.

The clinical specificity of **eSens Candida albicans/glabrata/krusei 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

	Catalogue number		Caution
	Batch code		Contains sufficient for <n> tests
	In vitro diagnostic medical device		Use-by Date
	Version		Consult instructions for use
	Temperature limit		Keep away from sunlight
	Manufacturer	C-	Negative control of extraction
	Date of manufacture	C+	Positive control of amplification
	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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