Candida Species RUO PCR

REF #: OLM2007-RUO

FOR RESEARCH USE ONLY. Not for use in diagnostic procedures. **qPCR kit for the detection of *Candida* species**

INTENDED PURPOSE

The *Candida* Species RUO PCR test kit is a multiplex real-time PCR assay for the detection of DNA from six *Candida* species (*C. albicans, C. glabrata, C. parapsilosis, C. tropicalis, C. krusei,* and *C. dubliniensis*).

BIOLOGICAL PRINCIPLES

REAL-TIME PCR

Individual primer and probe designs for detection of six clinically relevant *Candida* species *(C. albicans, C. glabrata*, *C. parapsilosis, C. tropicalis, C. krusei* and *C. dubliniensis)*, have been combined into 2 distinct 4-plex assays and their DNA can be detected through the different fluorescent channels as described in the kit contents.

IMMY's *Candida* Species RUO PCR test kit makes use of the most widely used qPCR chemistry, which is based on the detection of light emitted by hydrolysis probes (Figure 1).

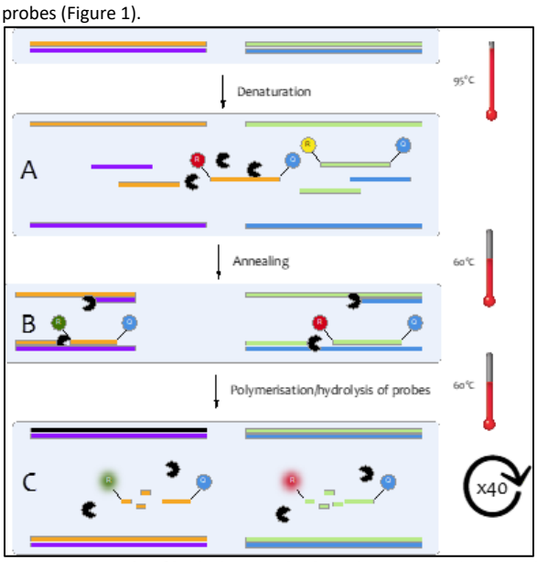

Figure 1. Principle of hydrolysis probe-based test. A. Double stranded genomic DNA, represented by the orange and purple strands, is denatured as the sample is briefly heated to 95°C. If added prior to the extraction of DNA, the assay also contains an internal extraction control, represented by blue and green strands. **B.** During the annealing phase of the PCR the temperature is reduced and forward and reverse primers (short colored lines) hybridize to any complementary target DNA present. The same reaction mixture also contains fluorogenic probes, which consist of target-specific DNA oligonucleotides labelled with a 5`-fluorescent reporter dye (R) and a quencher (Q). In the case of IMMY's *Candida* Species RUO PCR test kit, these probes are specific for (i) *C. albicans* (FAM), *C. glabrata* (HEX), *C. parapsilosis* (Cy5) and the internal extraction control (ROX), using the *Candida* Species RUO PCR premix (RED cap), and (ii) *C. tropicalis* (FAM), *C. krusei* (HEX), *C. dubliniensis* (Cy5) and the internal extraction control (ROX), using the *Candida* Species RUO PCR PLUS premix (BLUE cap). **C.** During the polymerization phase of the PCR, hybridized bound probes are displaced and cleaved by the 5' nuclease activity of the *Taq* polymerase (black circles), resulting in the physical separation of the reporters and quenchers. This results in the emission of light at a fluorophore-specific wavelength, which can be detected in the appropriate channels of a qPCR instrument.

POSITIVE CONTROL (PC)

The kit contains 2 vials of positive control, each containing a template specifying three *Candida* species targets, corresponding to the *Candida* Species RUO PCR and *Candida* Species RUO PCR PLUS assay targets. The positive control is handled like a normal nucleic acid extract and indicates that the primers and probes for detecting *Candida* species are working properly in the run. Inclusion of the positive control depends on the preference of the end user and is not mandatory in every run. However, IMMY recommends its use in every run as it provides more confidence in results if all samples are negative. The positive control does not need to be subjected to a nucleic acid extraction procedure. Care should be taken to avoid cross-contamination of other samples when adding the positive control to the run. Any risk can be minimized by sealing all other samples and negative controls before pipetting the positive control into the positive control well.

NO TEMPLATE CONTROL (NTC)

To confirm the absence of contamination, at least one no template control (NTC) reaction must be included in every PCR run. For this reaction, RNase/DNase free water should be used instead of template.

INTERNAL EXTRACTION CONTROL (IEC)

The internal extraction control (IEC) is added to distinguish true negative samples from false negative samples, which can result from nucleic acid degradation, failure of nucleic acid extraction step, PCR inhibition, non-specific probe hydrolysis or qPCR instrument malfunction. The primers and probe necessary to detect the IEC are included in the multiplex primer and probe mix, for both *Candida* Species RUO PCR and *Candida* Species RUO PCR PLUS. Therefore, a single DNA extraction can be performed for a research sample, and the extract tested in parallel in the *Candida* Species RUO PCR and *Candida* Species RUO PCR PLUS assays. The IEC template is added either to the DNA lysis/extraction buffer or to the sample once it has been resuspended in lysis buffer. The IEC will give a quantification cycle (Cq) value of >25, with the variation depending on the efficiency of sample extraction and level of sample dilution.

REAGENTS PROVIDED

Each kit contains sufficient reagents for 50 tests.

<table>
<tr><td>Candida Species RUO PCR Primer/Probe Mix
FAM, HEX, Cy5, and ROX Labeled (see below)
<table>
<tr><th>Target</th><th>Fluorophore</th><th>Absorption (nm)</th><th>Emission (nm)</th></tr>
<tr><td>C. albicans</td><td>FAM</td><td>494</td><td>518</td></tr>
<tr><td>C. glabrata</td><td>HEX</td><td>535</td><td>556</td></tr>
<tr><td>C. parapsilosis</td><td>Cy5</td><td>646</td><td>669</td></tr>
<tr><td>Internal extraction control</td><td>ROX</td><td>575</td><td>602</td></tr>
</table></td><td>> 100 µL</td></tr>
<tr><td>Candida Species RUO PCR PLUS Positive Control
C. albicans/glabrata/parapsilosis</td><td>> 250 µL</td></tr>
<tr><td>Candida Species RUO PCR PLUS Primer/Probe Mix
FAM, HEX, Cy5, and ROX Labeled (see below)
<table>
<tr><th>Target</th><th>Fluorophore</th><th>Absorption (nm)</th><th>Emission (nm)</th></tr>
<tr><td>C. tropicalis</td><td>FAM</td><td>494</td><td>518</td></tr>
<tr><td>C. krusei</td><td>HEX</td><td>535</td><td>556</td></tr>
<tr><td>C. dubliniensis</td><td>Cy5</td><td>646</td><td>669</td></tr>
<tr><td>Internal extraction control</td><td>ROX</td><td>575</td><td>602</td></tr>
</table></td><td>> 100 µL</td></tr>
<tr><td>Candida Species RUO PCR PLUS Positive Control
C.tropicalis/C. krusei/C.dubliniensis</td><td>> 250 µL</td></tr>
<tr><td>Internal Extraction Control DNA (IEC)</td><td>> 500 µL</td></tr>
<tr><td>qPCR Master Mix</td><td>> 1000 µL</td></tr>
<tr><td>RNase/DNase-Free Water</td><td>> 800 µL</td></tr>
</table>

Refer to the Safety Data Sheets for more information on hazards and warnings.

MATERIALS REQUIRED BUT NOT PROVIDED

- Disposable gloves
- Protective glasses
- DNA extraction kit
- Pipette(s) and associated disposable tips
- 1.5 mL microcentrifuge tubes
- Vortex mixer
- Centrifuge
- qPCR plate and plate seals
- qPCR instrument
- Biohazard waste receptacle

REAGENT STABILITY AND STORAGE

The entire *Candida* Species RUO PCR test kit should be stored at -20 °C until the expiration date printed on the product label (18 months from date of manufacture). Components should be kept at 0 °C, not be exposed to temperatures above -20 °C for longer than 30 minutes at a time and unnecessary repeated freeze/thawing should be avoided. Please note that exposure to light leads to photo bleaching of the fluorescent reporters and so reduces the sensitivity of the assay.

The quality of the product cannot be guaranteed after the expiration date.

REAGENT PRECAUTIONS

1. IMMY cannot guarantee the performance of its products when used with materials purchased from other manufacturers. Do not interchange reagents from different kit lot numbers or other manufacturers.
2. The user assumes full responsibility for any modification to the procedures published herein.
3. Avoid reagent contamination by following contamination control practices for PCR and segregating workflow as appropriate.
4. Always ensure reagents are defrosted thoroughly, mixed, spun down, and kept on ice.

5. A separate PCR premix must be prepared for each of the 3-plex assays (*Candida* Species RUO PCR and *Candida* Species RUO PCR PLUS).
6. Do not mix the 2 Primer/Probe reagents provided in this kit.
7. Ensure the appropriate Positive Control is used with its corresponding assay (Tubes are color- coded).
8. Do not load the 2 different premixes into the same reaction well of your PCR plate.
9. As both assays (*Candida* Species RUO PCR and *Candida* Species RUO PCR PLUS) operate in the same fluorescence channels, please ensure the wells of your reaction plate are labelled appropriately, to differentiate between respective assay targets.
10. Do not use kit or any kit reagents after the stated expiration date.

WARNINGS AND PRECAUTIONS FOR USERS

1. Wear protective clothing, including lab coat, eye/face protection, and disposable gloves, and handle the kit reagents and research samples using standard precautions. Wash hands thoroughly after performing the test.
2. Avoid splashing samples or solutions.
3. Biological spills should be wiped thoroughly with an effective disinfectant. Disinfectants that can be used include (but are not limited to) a solution of 10% bleach, 70% ethanol, or 0.5% Wescodyne Plus™. Materials used to wipe up spills may require biohazardous waste disposal.
4. Dispose of all samples and materials used to perform the test as though they contain an infectious agent. Laboratory chemical and biohazardous wastes must be handled and discarded in accordance with all local, regional, and national regulations.
5. Standard precautions and local laboratory guidelines should be followed.
6. Low concentrations of DNA can be unstable if stored for long periods. IMMY advises that sample storage time should be minimized before testing.
7. Ensure all additional required consumables are RNase/DNase-free.
8. Use disposable filter tips for all pipetting.
9. Thaw DNA samples on ice and keep on ice.
10. Safety Data Sheets are available upon request.

SAMPLES

DNA should be extracted from culture or research derived samples. Extracted samples should be stored between -80°C and -20°C for long-term storage. IMMY *Candida* Species RUO PCR kits are designed for use with research samples prepared using nucleic acid extraction methods compatible with PCR amplification.

Successful amplification depends on the quality of nucleic acids in the sample. Samples with degraded DNA, low concentration, or PCR inhibitors may yield invalid or negative results. Always run at least one negative (no template) control with the samples. To prepare a negative-control, replace the template DNA sample with RNase/DNase-free water.

PROCEDURE

1. Remove the test kit from the freezer and allow the reagents to thaw. Thawed reagents must be kept on ice. Briefly vortex and centrifuge all reagent tubes prior to use.

DNA EXTRACTION

1. The IEC DNA can be added either to the DNA lysis/extraction buffer or to the research sample once it has been resuspended in lysis buffer.
   NOTE: DO NOT add the Internal Extraction Control DNA directly to the unprocessed biological sample as this will lead to degradation and a loss in signal.
2. Add 5 µL of the IEC DNA to each sample in DNA lysis/extraction buffer for elution into 50 µL. For eluting into different volumes adjust volume of the IEC DNA accordingly.
3. Complete the DNA extraction according to the manufacturer's recommended protocols.

PCR DETECTION PROTOCOL

1. Prepare a PCR premix according to the table below. Ensure that *Candida* Species RUO PCR and *Candida* Species RUO PCR PLUS premixes are prepared separately. Volumes are given per reaction well and should be multiplied by the number of reaction wells required, include sufficient reactions for positive and negative controls. The prepared PCR premix should be thoroughly mixed and briefly spun down.

Component	Volume/Reaction
qPCR Master Mix	10 µL
Primer/Probe Mix	2 µL
RNase/DNase-Free Water	2 µL
Final Volume	14 µL

Due to small variations in pipetting accuracy, we recommend that you allow an additional 10% for the final volume, i.e. if you are assaying ten samples, make up sufficient reaction mix for 11 tests.

2. Pipette 14 µL of prepared PCR premix into each reaction well according to your qPCR experimental plate set up.
3. Prepare sample DNA templates for each of your samples (see DNA extraction step).
4. Pipette 6 µL of DNA template into each well according to your experimental plate set up.
5. For negative control wells use 6 µL of RNase/DNase-free water. For positive control use 6 µL of *Candida* Species RUO PCR/*Candida* Species RUO PCR PLUS Positive Control, as appropriate. The final volume in each well is 20 µL.
6. Ensure that your PCR reaction plate is sealed and briefly centrifuged before transferring to a validated thermocycler for amplification.

AMPLIFICATION PROTOCOL

1. If using an instrument that uses ROX as a passive reference then the passive reference must be turned off or set to "none" for no passive reference, as the IEC uses the ROX channel.
2. Please refer to your instrument manual for instructions on setting up an amplification run. Amplification should be carried out according to the conditions detailed the table below.

	Step	Time	Temp
Cycling x40	Enzyme Activation	2 mins	95 °C
	Denaturation	5 secs	95 °C
	Data Collection*	20 secs	60 °C

** Fluorogenic data should be collected during this step through the FAM, HEX, ROX, and Cy5 channels*

RESULTS

Refer to the instruction manual for your thermocycler for information on how to operate the qPCR instrument and perform data analysis. Both *Candida* Species RUO PCR and *Candida* Species RUO PCR PLUS assays operate in the FAM, HEX, ROX and Cy5 channels. Therefore, appropriate labelling of the reaction plate wells is required. Remember to apply color compensation if appropriate to your qPCR instrument.

It is important to visually inspect the amplification plots for each sample to ensure that the results recorded are due to true amplification and cannot be attributed to background noise recorded above the defined thresholds.

NOTE: Cq estimations stated below are calculated with threshold positioned at the center of the log- linear range of the PCR amplification curve.

POSITIVE CONTROL (PC)

The supplied positive controls should each record characteristic amplification plots through the FAM, HEX and Cy5 channels, with Cq of 28 ± 2. There is no internal control template within the positive control so the ROX channel should record no signal (flat amplification plots). Each positive control generates signal to indicate that the assay is working correctly for detection of three target *Candida* species.

NO TEMPLATE CONTROL (NTC)

The NTC should give a flat line (flat amplification plots) through all channels. Signals in the NTC may indicate cross contamination during plate set up.

SAMPLE DATA

1. Successful sample extraction is indicated by a positive signal in the ROX channel (Cq>25), with lower Cqs indicative of more efficient extraction.
2. Presence of a signal in the FAM/HEX/Cy5 channel indicates that the sample contains C. albicans/glabrata/parapsilosis (for *Candida* Species RUO PCR), and C tropicalis/krusei/dubliniensis (for *Candida* Species RUO PCR PLUS), respectively. Presence of signal in more than one of these channels indicates a mixed infection (mixed Candida species).
3. It is possible to obtain a signal in the FAM/HEX/Cy5 channel without also recording a signal in the ROX channel. Typically, this occurs when a high target load results in early amplification (i.e. low Cqs) and the accumulation of target DNA inhibits the amplification of the IEC DNA.
4. If there is no signal in any of the channels, the assay has failed and no conclusions are possible.

Candida Species RUO PCR**: Detection Channels**				
FAM	**HEX**	**Cy5**	**ROX**	**Result**
+	-	-	+	*C. albicans POSITIVE sample. IEC PASS.* ***Valid result***.
-	+	-	+	*C. glabrata POSITIVE sample. IEC PASS.* ***Valid result***.
-	-	+	+	*C. parapsilosis POSITIVE sample. IEC PASS.* ***Valid result***.
-	-	-	-	**Invalid result**.

Candida Species RUO PCR **PLUS: Detection Channels**				
FAM	**HEX**	**Cy5**	**ROX**	**Result**
+	-	-	+	*C. tropicalis* POSITIVE sample. IEC PASS. **Valid result**.
-	+	-	+	*C. krusei* POSITIVE sample. IEC PASS. **Valid result**.
-	-	+	+	*C. dubliniensis* POSITIVE sample. IEC PASS. **Valid result**.
-	-	-	-	**Invalid result**.

LIMITATIONS OF THE PROCEDURE

1. For research use only. Not for use in diagnostic procedures.

INTERNATIONAL SYMBOL USAGE

	Manufactured by		Lot Number
	Reference Number		Expiration Date
	Sufficient for “#” Tests		Research Use Only
	Consult Instructions for Use		Irritant

Rev. Date 2025-11-24
Rev. 1
For a list of IFU changes, email info@immy.com

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