

**ASSAY NAME: Candidiasis Panel (CAPN)**

**Quantity: 100 x 20µL PCR reactions**

**5-plex assay: *Candida albicans*, *Candida glabrata*, *Candida krusei*, *Candida lusitaniae*, and human RPP30 DNA**

**SKU #: BUN-CAPN-D-QS-100 (QuantStudio)**

**(RUO). Research Use Only. Not for use in Diagnostic Procedures.**

**SCOPE OF THIS DOCUMENT:**

The oligonucleotide recipes are optimized for QuantStudio. The verification data presented in this product information sheet were performed using PNP-CAPN-D-QS-100 on a QuantStudio™ 7 Flex Real-Time System. The performance on other QuantStudio instruments should be similar. Contact PCRassays.com if you are planning to use a different instrument.

**CONTENTS**

The primers and probes in the CAPN assay are provided in Tube 1 as a 5X concentrated working solution that detects 4 pathogens and a human control.

**Table of Dyes used in this assay:**

Pathogen/Target	Dyes	Quencher	Refs.
<i>C. krusei</i>	FAM	BHQ-1	1,2
<i>C. lusitaniae</i>	HEX	BHQ-1	3
RPP30-DNA control	TAMRA	BHQ-1	4
<i>C. albicans</i>	TEX615	BHQ-2	5, 6
<i>C. glabrata</i>	Cy5	BHQ-2	7

The probes are designed as TaqMan<sup>®</sup> cleavage mechanism and thus the reaction requires a DNA polymerase with 5'-exonuclease activity.

**ASSAY HANDLING AND CONTAMINATION**

The CAPN assay is shipped at ambient temperature, and should be stored at -20 °C. The assay should be kept on ice once thawed. Do not subject the enzyme to multiple freeze-thaw cycles.

Contamination should be avoided by using appropriate personal protective equipment (PPE), powder free gloves, aerosol barrier pipette tips, and a clean hood.

**ASSAY CONTENTS:**

**Tube 1:** 5X Primer/Probe mix for *Candida albicans*, *Candida glabrata*, *Candida krusei*, *Candida lusitaniae*, and hRPP30DNA.

**Tube 2: (Do NOT add to specimen unknowns)** Positive control: 5000 copies/µl of synthetic 500 bp DNA fragments for *C. albicans*, *C. glabrata*, *C. krusei*, *C. lusitaniae*, and hRPP30DNA.

**Tube 3:** InhibiTa<sup>q</sup> qPCR enzyme Mastermix (enough for 100 rxns. with 20 µL total volume). This is a custom formulation from Fortis Life Sciences to the specifications of PCRassays.com.

**Tube 4:** (Optional) Spike-in control. 1.0E6 copies/uL of synthetic 500 BP human RPP30 gene. **Do not add directly to the PCR reaction!**



**EXPERIMENTAL**

(Optional) add 1 µL of spike-in control (Tube 3) to each specimen before extraction. **Do not add directly to the PCR reaction!** It serves as extraction and PCR reaction control.

Perform nucleic acid extraction/purification (recommended).

Set up your PCR reaction (20 µL) as follows on ice:

Component	Volume (µL)
InhibiTa <sup>q</sup> enzyme mastermix (2X)	10
Primer/Probe mix (5X)	4
Sample	2
Water	4

**Notes: To improve assay sensitivity, up to 6 µL of sample can be added (water volume adjusted accordingly) for a total reaction volume of 20 µL. For positive control rxns., add 2 µL of the solution from Tube 2. Molecular biology grade water should be used to prepare the PCR reactions, which is NOT included in this assay.**

A PCR protocol was used for verification on a QuantStudio™ 7 Flex Real-Time System, with the following program (this protocol is designed to minimize formation of air bubbles that are a common feature on QuantStudio instruments):

Step	Thermocycling Protocol:
1	Incubate @ 85 °C for 1 second
2	Incubate @ 94 °C for 2 minutes
3	Incubate @ 94 °C for 3 seconds
4	Incubate @ 55 °C for 22 seconds
5	Plate Read
6	Incubate @ 85 °C for 1 second
7	Incubate @ 94 °C for 3 seconds
8	Incubate @ 55 °C for 22 seconds
9	Plate Read
10	Go to Step 6, repeat 43x more

## RESULT INTERPRETATION

After running the qPCR reaction, perform a regression analysis on the data to determine the quantification cycle, C<sub>q</sub>. (C<sub>q</sub> is preferred over Ct). Each fluorescence channel with a C<sub>q</sub> < 38 cycles and final RFU > 200,000 is considered “positive” or “+” in the Table below.

<i>C. krusei</i> FAM™	<i>C. lusitanae</i> HEX™	<i>C. albicans</i> TEX615™	<i>C. glabrata</i> CY5™	hRPP30 TAMRA™	Recommended Interpretation
–	–	–	–	–	The PCR reaction failed. Please repeat the experiment
–	–	–	–	+	The sample does not contain bacterial DNA of interest. The sample contains human RPP30 DNA.
+	–	–	–	–	The sample contains <i>C. krusei</i> DNA. The sample may not contain human RPP30 DNA.
+	–	–	–	+	The sample contains <i>C. krusei</i> DNA and human RPP30 DNA.
–	+	–	–	–	The sample contains <i>C. lusitanae</i> DNA. The sample may not contain human RPP30 DNA.
–	+	–	–	+	The sample contains <i>C. lusitanae</i> DNA and human RPP30 DNA.
–	–	+	–	–	The sample contains <i>C. albicans</i> DNA. The sample may not contain human RPP30 DNA.
–	–	+	–	+	The sample contains <i>C. albicans</i> DNA and human RPP30 DNA.
–	–	–	+	–	The sample contains <i>C. glabrata</i> DNA. The sample may not contain human RPP30 DNA.
–	–	–	+	+	The sample contains <i>C. glabrata</i> DNA and human RPP30 DNA.
+	+	+	+	–	The sample contains <i>C. krusei</i> , <i>C. lusitanae</i> , <i>C. albicans</i> , <i>C. glabrata</i> DNA. The sample may not contain human RPP30 DNA.
+	+	+	+	+	The sample contains <i>C. krusei</i> , <i>C. lusitanae</i> , <i>C. albicans</i> , <i>C. glabrata</i> DNA and human RPP30 DNA.

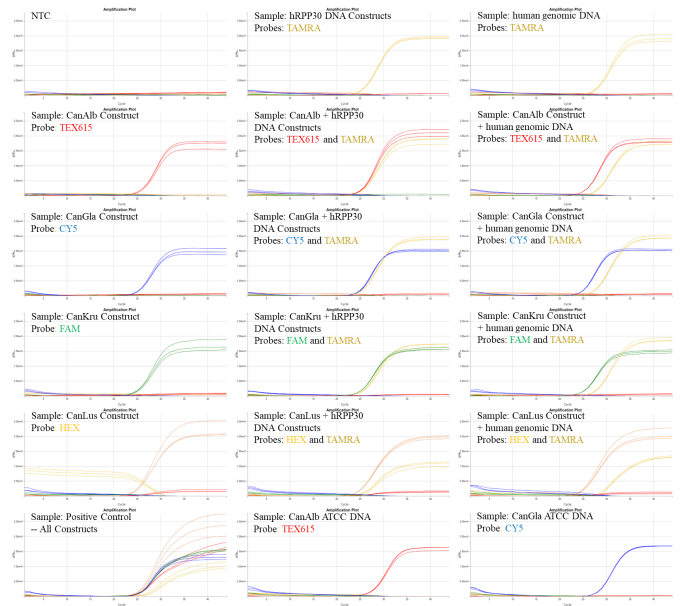
## VERIFICATION EXPERIMENTS

The CAPN assay verification was carried out as a 5-plex assay, which simultaneously detects DNA from *C. albicans*, *C. glabrata*, *C. krusei*, *C. lusitanae*, and human RPP30 DNA, which serves as a positive extraction-control assay.

Experiments were performed in triplicate using the experimental procedure given above, but with different samples added to each reaction. The samples used for the verification experiments contained 1×10<sup>4</sup> copies/reaction of synthetic 500 bp synthetic DNA constructs (from Twist Biosciences) harboring the regions of interest from the pathogen genomes, human RPP30 DNA gene, and human genomic DNA. The results of these experiments are shown in **Figure 1** and indicate that the 5-plex specifically detects the different bacterial species in the human genomic DNA matrix. Two standard DNA extract samples from ATCC of *C. albicans* and *C. glabrata* were tested with CAPN assay, and the results are positive.

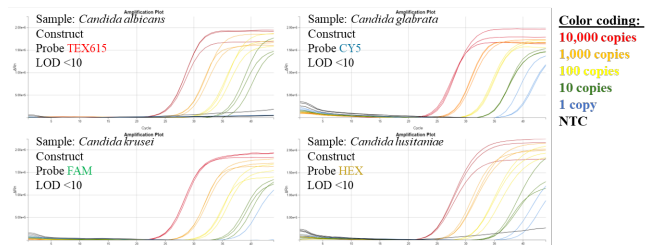
### NOTES

- <sup>1</sup> FAM™ (Carboxyfluorescein), a trademark of Life Technologies, Inc
- <sup>2</sup> BHQ-1™ (Black Hole Quencher) is a trademark of Biosearch Technologies, Inc.
- <sup>3</sup> HEX™ (Hexachloro-fluorescein), a trademark of Applera Corp.
- <sup>4</sup> TAMRA (Carboxytetramethylrhodamine), a trademark of Applera Corp.
- <sup>5</sup> TEX615™ is a trademark of Thermo Fisher Scientific.
- <sup>6</sup> BHQ-2™ (Black Hole Quencher) is a trademark of Biosearch Technologies, Inc.
- <sup>7</sup> Cy5™, a trademark of GE Healthcare.
- <sup>8</sup> “TaqMan” is a trademark of Roche Molecular Systems, Inc.



**Figure 1:** Verification experiments with single targets (given in text boxes for each panel). All sets of probes and primers are present in every reaction, but positive signal is only observed when the target(s) is present, indicating that the amplification is specific.

The limit of detection (LOD) was estimated by performing serial dilution experiments in triplicate (**Figure 2**). For dilution series only target construct was added. The results show a limit of detection (LOD) < 10 copies/reaction.



**Figure 2:** Serial dilution experiments show LOD < 10 molecules for the synthetic DNA construct of each target.

**Conclusion:** The data in **Figure 1** indicate that the 5-plex primers and probes specifically detect and differentiate the bacterial types and are also compatible with RPP30\_DNA positive control primers. Human genomic DNA matrix doesn't affect detection of pathogen DNA.

### CONTACT US

For assistance, please contact DNA Software using the link: <https://www.pcrassays.com/contact/>

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