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Produktinformation

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Lieferung & Zahlungsart

siehe unsere Liefer- und Versandbedingungen

Zuschläge

- Mindermengenzuschlag
- Trockeneiszuschlag
- Gefahrgutzuschlag
- Expressversand

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PCRassays.com

ASSAY NAME: CAN1

Quantity: 100 x 20µL PCR reactions

5-plex assay: *Candida glabrata*, *Candida guilliermondii, Candida parapsilosis, Candida tropicalis*, and human RPP30 DNA

SKU#: PNP-CAN1-D-BR-100 (Bio-Rad)
PNP-CAN1-D-QS-100 (QuantStudio)

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

SCOPE OF THIS PRODUCT INFORMATION SHEET:

The oligonucleotide recipes are optimized for each instrument (BioRad, QuantStudio). The verification data presented in this IFU were performed using PNP-CAN1-D-BR-100 on a Bio-Rad CFX 96. The performance of the other SKUs on their corresponding instrument should be similar. Contact PCRassays.com if you are planning to use a different instrument.

CONTENTS

The primers and probes in the Candida M1 (CAN1) 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.
C. parapsilosis	FAM	BHQ-1	1,2
RPP30-DNA control	HEX	BHQ-1	7
C. tropicalis	TEX615	BHQ-2	3,4
C. glabrata	Cy5	BHQ-2	5
C. guilliermondii	Cy5.5	BHQ-2	6

The probes are designed as TaqMan[8] cleavage mechanism and thus the reaction requires a DNA polymerase with 5'-exonuclease activity.

Note: molecular biology grade water should be used to prepare the PCR reactions, which is NOT included in this assay.

ASSAY CONTENTS:

Tube 1: 5X Primer/Probe mix for *C. parapsilosis, C. tropicalis*, *C. glabrata*, *C. guilliermondii,* and hRPP30DNA.

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

Tube 3: Spike-in control. 1.0E6 copies/uL of synthetic 500 BP human RPP30 gene. **Do not add directly to the PCR reaction!**

Tube 4: InhibiTaq 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.

ASSAY HANDLING AND CONTAMINATION

The CAN1 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.

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

EXPERIMENTAL

(Optional) add 1 µL of spike-in control (Tube 3) to the 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)
InhibiTaq 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 (i.e., the "sample").

A PCR protocol was used in-house for verification on a Bio-Rad CFX96™ Real-Time System, with the following program:

Step	Thermocycling Protocol:
1	Incubate @ 95 °C for 2 minutes
2	Incubate @ 95 °C for 3 seconds
3	Incubate @ 55 °C for 15 seconds
4	Plate Read
5	Go to Step 2, repeat 44xmore

For QuantStudio instruments, we recommend a step 3 cycle time of 22 seconds at 55 °C.

RESULT INTERPRETATION

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

C.parap. FAM™	*C.tropical.* TEX615™	*C.glabrata* Cy5™	*C. guillier.* Cy5.5™	hRPP30 HEX™	Recommended Interpretation
–	–	–	–	–	The PCR reaction failed. Please repeat the experiment
–	–	–	–	+	The sample does not contain DNA of interest. The sample contains human RPP30 DNA.
+	–	–	–	–	The sample contains *C. parapsilosis* DNA. The sample may not contain human RPP30 DNA.
+	–	–	–	+	The sample contains *C. parapsilosis* DNA and human RPP30 DNA.
–	+	–	–	–	The sample contains *C. tropicalis* DNA. The sample may not contain human RPP30 DNA.
–	+	–	–	+	The sample contains *C. tropicalis* DNA and human RPP30 DNA.
–	–	+	–	–	The sample contains *C. glabrata* DNA. The sample may not contain human RPP30 DNA.
–	–	+	–	+	The sample contains *C. glabrata* DNA and human RPP30 DNA.
–	–	–	+	–	The sample contains *C. guilliermondii* DNA. The sample may not contain human RPP30 DNA.
–	–	–	+	+	The sample contains *C. guilliermondii* DNA and human RPP30 DNA.
+	+	+	+	–	The sample contains *C. parapsilosis*, *C. tropicalis*, *C. glabrata*, *C. guilliermondii* DNA. The sample may not contain human RPP30 DNA.
+	+	+	+	+	The sample contains *C. parapsilosis*, *C. tropicalis*, *C. glabrata*, *C. guilliermondii* DNA and human RPP30 DNA.

VERIFICATION EXPERIMENTS

The CAN1 assay verification was carried out as a multiplexed assay, which simultaneously detects DNA of *C. glabrata, C. guilliermondii, C. parapsilosis, C. tropicalis,* 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 1×10^4 copies/reaction of synthetic 500 bp synthetic DNA constructs (from Twist Biosciences) harboring the regions of interest from the target genomes and the human RPP30 DNA gene, and human genomic DNA. The results of these experiments are shown in **Figure 1**:

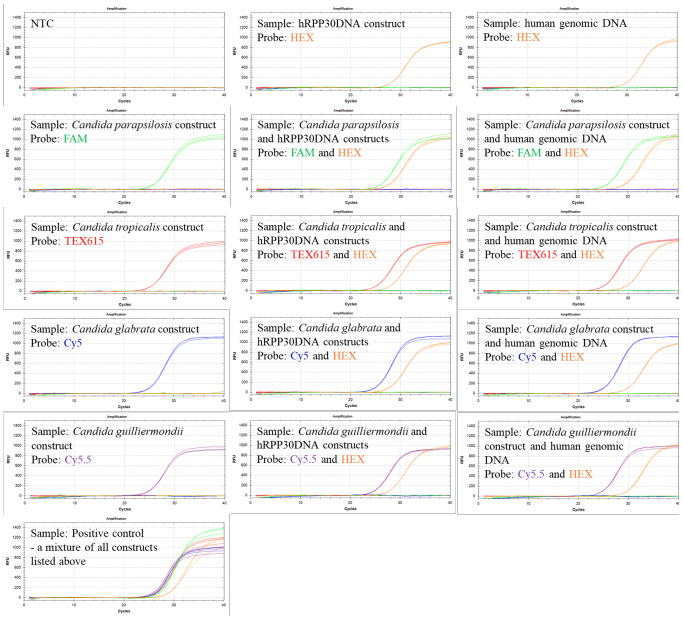

Figure 1: CAN1 verification experiments with single or double target(s) (given in text boxes for each panel). All sets of probes and primers are present in every reaction, but positive signal is only observed when the target(s) is present, indicating that the amplification is specific.

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

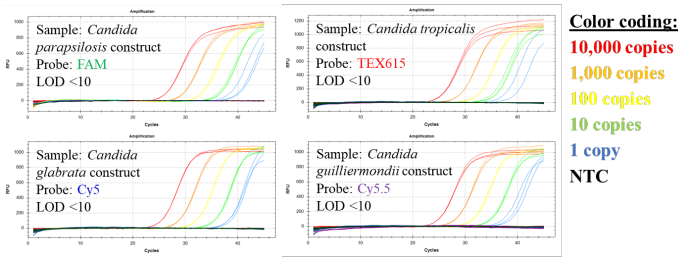

Figure 2: CAN1 serial dilution experiments show LOD <10 molecules for the synthetic DNA construct of each target.

Conclusion: The data in **Figure 1** indicates that the *C. parapsilosis, C. tropicalis, C. glabrata, C. guilliermondii,* primers and probe are compatible with DNAS RPP30 DNA positive control primers and probe in the human genomic DNA matrix.

CONTACT US

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

Address: Michigan Life Science and Innovation Center,
46701 Commerce Center Dr, Plymouth, MI 48170

Phone: (734) 222-9080

NOTES

[1] FAM™ (Carboxyfluorescein), a trademark of Life Technologies, Inc

[2] BHQ-1™ (Black Hole Quencher) is a trademark of Biosearch Technologies, Inc.

[3] TEX615™ is a trademark of Thermo Fisher Scientific.

[4] BHQ-2™ (Black Hole Quencher) is a trademark of Biosearch Technologies, Inc.

[5] Cy5™, a trademark of GE Healthcare.

[6] Cy5.5™, a trademark of Amersham Biosciences Corp

[7] HEX™ (Hexachloro-fluorescein), a trademark of Applera Corp.

[8] "TaqMan" is a trademark of Roche Molecular Systems, Inc