Kit: C.krusei-RPP30DNA
Quantity: 100 x 20µL PCR reactions
Duplexed assay: *Candida krusei* and human genomic RPP30 DNA
Gene: Protein ID: XP_029322976.1
Cat #: CANKRU-RPP30DNA-TC-0053

Kit contents:
Tube 1: 20X Primer/Probe mix for *Candida krusei* and hRPP30
Tube 2: 5000 copies/µl Positive controls of synthetic DNA for both *C. krusei* and hRPP30.

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

CONTENTS

The C.krusei-RPP30DNA kit contains a mixture of primers/probe targeting an uncharacterized protein (Protein ID: XP_029322976.1), which is encoded in chromosome 4 (NC 042509.1) of the *Candida krusei* genome (also known as *Pichia kudriavzevii*). The primers and probes in Tube 1 are provided as a 20X concentrated working solution. The fluorophore of the *Candida krusei* probe is FAM™ and the quencher is BHQ-1™.[1,2] The same mix also contains primers/probe targeting human RPP30DNA Intron I (20X concentrated) as a PCR positive control assay for human samples. The fluorophore of the RPP30DNA probe is HEX™ and the quencher is BHQ-1™.[3] The probes are designed as TaqMan[4] cleavage mechanism and thus the reaction requires a DNA polymerase with 5'-exonuclease activity (we recommend InhibiTaq PLUS qPCR Master Mix, from Empirical Biosciences, Cat number: ITMP-MM-2500).

Tube 2 contains a mixture of two synthetic 500 bp DNA constructs containing the amplicon regions of *Candida krusei* and a region of hRPP30 that is provided as a positive extraction control. The concentration of each DNA construct is approximately 5,000 copies/µL. The Control DNA constructs are for validation purposes only and **Tube 2 should NOT be added to wells for specimen unknowns**.

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

KIT HANDLING AND CONTAMINATION

The C.krusei-RPP30DNA kit is shipped at ambient temperature, and should be stored at -30 to -15°C. The kit should be kept on ice once thawed.

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

EXPERIMENTAL

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

Component	Volume (µL)
InhibiTaq mastermix (2X)	10
Primer/Probe mix (20X)	1
Sample	2
Water	7

Note: The composition of this reaction is calculated based on the user manual of InhibiTaq PLUS qPCR Master Mix, from Empirical Biosciences. The volume of water should be adjusted accordingly if the user's reaction preparation is different from the recommended preparation method.

A PCR protocol was used at DNA Software, Inc. for pre-validation 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 30 seconds
4	Plate Read
5	Go to Step 2, repeat 44x more
7	(optional) Incubate @55 °C for 3 minutes

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.

Candida krusei (FAM™)	RPP30DNA (HEX™)	Recommended Interpretation
–	–	The PCR reaction failed. Please repeat the experiment.
–	+	The sample does not contain *Candida krusei* DNA.
+	–	The sample contains *Candida krusei* DNA. The sample may not contain human RPP30 DNA.
+	+	The sample contains *Candida krusei* DNA and human RPP30 DNA.

PRE-VALIDATION DATA

The C.krusei-RPP30DNA kit validation was carried out as a duplexed assay, which simultaneously detects DNA from *Candida krusei* and DNA from the human RPP30DNA gene, 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 validation experiments contained 1×10^4 copies/reaction of synthetic 500 bp synthetic DNA constructs (from Twist Biosciences) harboring the regions of interest from *Candida krusei* genome and the RPP30DNA gene. The results of these experiments are shown in **Figure 1** below:

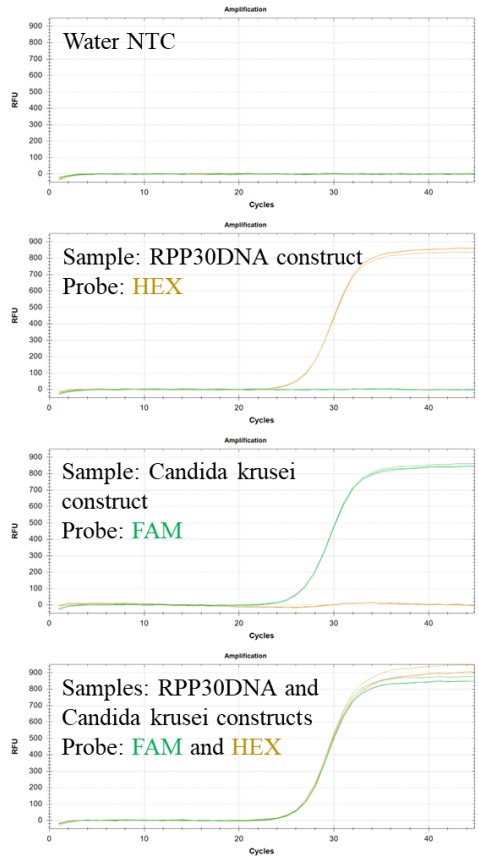

Figure 1: Validation experiments with single or double target(s) (given in text boxes for each panel). Both sets of probes and primers are present in every reaction, but positive signal is only observed when the target(s) is present, indicating that the amplification is specific. The **FAM** probe detects *Candida krusei* construct DNA. The **HEX** probe detects RPP30DNA construct DNA.

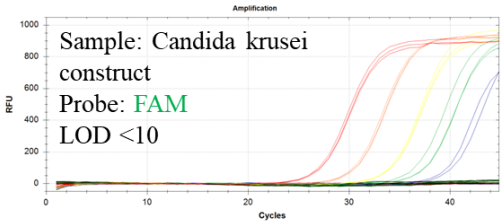

Figure 2: Serial dilution experiments show LOD <10 molecules for *Candida krusei* 500 bp synthetic DNA construct.

Conclusion: The data in **Figure 1** indicate that the *Candida krusei* primers and probe are compatible with DNAS RPP30DNA positive control primers and probes in a 2-plex application to detect *Candida krusei* in the matrix of human sample extract.

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

CONTACT US

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

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NOTES

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

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

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

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