YOUSEQ

CRYPTOCOCCUS NEOFORMANS

qPCR TEST HANDBOOK

For Research Use Only. Not intended for diagnostic use.

CAT NO.: YSL-qP-EC-C.neofo-100

100 reactions
with Endogenous Control and Lyophilised MasterMix

VERSION 8.5

YOUSEQ

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INTENDED USE

This qPCR test offers an efficient and user-friendly solution for the detection of Cryptococcus neoformans (C.neofo) in extracted nucleic acid samples from a variety of sources. It is intended for use by trained professionals in a suitable molecular biology laboratory.

SPECIFICITY AND SENSITIVITY

Specificity

The YouSeq qPCR test for detection of Cryptococcus neoformans is designed to have the broadest detection profile possible and detect all clinically relevant strains. The primers and probes typically have a ≥95% homology with all reference data used, from relevant, publicly available databases at the time of design.

The target gene for Cryptococcus neoformans (18s rRNA biogenesis protein (RCL1) gene) has been demonstrated to have a distinctive sequence making it an ideal target for highly specific detection.
The primers will detect all Cryptococcus neoformans strains. However due to extreme homology it is possible that the kit may also detect some strains of Cryptococcus deneoformans.

This qPCR kit is also known as Filobasidiella neoformans.

For further information on the detection profile of the product, please do not hesitate to contact our team: support@youseq.com

Sensitivity

The qPCR test is suitable for the detection of Cryptococcus neoformans, across a wide dynamic range. Under ideal PCR conditions the assay can detect less than 100 copies of the target in the PCR reaction.

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Component	Cap Colour	Volume
Cryptococcus neoformans specific primer/probe mix (FAM)		100 µL
Endogenous Control primer/probe mix (VIC/HEX)		100 µL
Lyophilised Tetra™ 2X qPCR MasterMix		1.1 mL*
MasterMix Resuspension Buffer (MMRB)		1.5 mL
Cryptococcus neoformans Positive Control (PTC)		500 µL*
Template Resuspension Buffer (TRB)		1.5 mL
DNase/RNase Free Water		1.5 mL
ROX Passive Reference Dye		10 µL

* Supplied dried – requires resuspension. See instructions in resuspension section.

RECOMMENDED ADDITIONAL REAGENTS & MATERIALS

Nucleic Acid extraction kit

General laboratory equipment (pipettes, pipette tips, (micro)centrifuge tubes, compatible strip tubes/plates, plate seals, etc.).

qPCR instrument with channels to detect FAM and VIC/HEX.

BEST PRACTICE

Decontamination:

Before beginning laboratory work, thoroughly decontaminate any work surfaces and pipettes being used, to eliminate potential contamination.

General use and set-up:

All components should be fully defrosted with contents at the bottom of the tube before opening. To ensure contents are at the bottom, centrifuge or gently tap the tube. After use, reagents should be returned to the freezer.

Once any reagents are resuspended, mark the tick box on the tube for future reference. After this, or after combining reagents, the tube should be pulse vortexed to ensure it is mixed well.

It is advised to set up the tubes/plate and reaction mix on ice to minimise artefact formation, which may reduce sensitivity.

When preparing the qPCR reaction mix, it is recommended to incorporate an overage when calculating the total number of reactions to compensate for potential volume losses incurred during pipetting.

Set-up environments:

It is best practice to set up qPCR tubes/plates in two different environments - a clean (no template) lab and PCR (template) lab.

No Template Control(s) (NTC) and Positive Control(s) (PTC) should be included in every run. To reduce contamination, NTCs and samples can be set up and sealed in a clean lab before moving to the PCR lab.

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BENCH SIDE PROTOCOL

RESUSPENSION

Before first use, resuspend the designated component with the correct reagent and specified volume, as per the table below:

1. Add the resuspension reagent and pulse vortex the tube to ensure each is mixed well.

Component	Reagent	Volume	Location
Lyophilised Tetra™ 2X qPCR MasterMix	MMRB	1.1 mL	Clean lab
Cryptococcus neoformans Positive Control (PTC)	TRB	500 µL	PCR lab

ROX (INSTRUMENT DEPENDENT)

ROX is required for instruments that use ROX as a passive reference. The table below outlines the qPCR instruments that require the addition of ROX.

1. If ROX is required:
    a. Dilute the ROX supplied according to the table below based on the intended qPCR instrument to be used.
    b. Directly transfer 5µL of prepared ROX to the resuspended Tetra™ MasterMix.

Level of ROX	Instruments	Step 1: Volume of water to add to ROX tube	Step 2: Add to MasterMix vial
High ROX:	Applied Biosystems 7000, 7300, 7700, 7900, StepOne, StepOne Plus	No Dilution Required	5 µL
Low ROX:	Applied Biosystems 7500 & 7500 FAST, ViiA7, Quantstudio, Stratagene MX	130 µL	5 µL
ROX Not Required:	All Other Instruments	Not Required	Not Required

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qPCR REACTION SET-UP

1. Retrieve the required components and appropriate plasticware for qPCR reaction set-up.

2. In an appropriately sized (micro)centrifuge tube, combine the following reagents to create a reaction mix that will cover all required tube/wells (e.g. samples, NTC and PTC).

Please note: When calculating required reactions, include an overage to allow for volume loss during pipetting

Component	Volume (per reaction)
Tetra™ 2X qPCR MasterMix	10 µL
Cryptococcus neoformans specific primer/probe mix	1 µL
Endogenous Control primer/probe mix	1 µL
Reaction mix volume	12 µL

3. Mix the combined reagents by briefly vortexing or inverting.

4. Dispense 12 µL of the reaction mix into all required tubes/wells.

5. For the NTC(s), add 8 µL of DNase/RNase Free Water into required tube/well(s).

6. For each extracted sample, add 8 µL into required tube/well(s).

Please note: It is best practice to seal the NTC(s) and sample tubes/wells before proceeding to the positive control steps.

7. For the positive controls(s), follow either step a. or b. below:

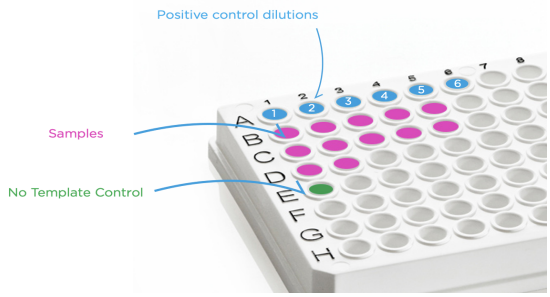
   a. For **qualitative** results, add 5 µL of the resuspended PTC (tube 1) and 3 µL of DNase/RNase Free Water, into each designated positive control tube/well. Proceed to step 8.

   b. For **quantitative** results, perform a serial dilution of the resuspended PTC (tube 1) to create a six-point standard curve:

      I. Add 45 µL of Template Resuspension Buffer into 5 fresh microcentrifuge tubes and label them 2, 3, 4, 5 and 6.
      II. Pipette 5 µL of PTC into tube 2.
      III. Mix by pipetting up and down 10 times.
      IV. Change pipette tip and pipette 5 µL from tube 2 into tube 3.
      V. Mix by pipetting up and down 10 times.
      VI. Repeat steps 'IV' and 'V' to complete the dilution process for tubes 4, 5 and 6.
      VII. Add 3 µL of DNase/RNase Free Water into each designated positive control tube/well.
      VIII. Add 5 µL of the required PTC dilution (tubes 1 – 6), into each designated positive control tube/well.

Please note: The described standard curve provides a dynamic range as outlined in the table below.

Tube No.	Copies of Target/reaction
1	1,000,000
2	100,000
3	10,000
4	1,000
5	100
6	10



8. Seal the tube(s)/plate.

9. Briefly vortex the sealed tube(s)/plate, then spin in a centrifuge to ensure all reagents are fully resuspended and at the bottom of the tubes/wells before proceeding.

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qPCR AMPLIFICATION PROTOCOL

1. Load the plate onto the qPCR instrument and set up the qPCR protocol following the table below.

2. Set the total reaction volume to 20 µL.

Temperature	Time	Number of Cycles
95°C	3 minutes	-
95°C	15 seconds	x 45
60°C	60 seconds	

Collect fluorogenic data through FAM and VIC/HEX channels during this step.

3. Start the run.

INTERPRETATION OF RESULTS – OVERVIEW

If using single threshold analysis – YouSeq recommends setting the threshold at 10% of the End Point Fluorescence (EPF) for each channel.

- For the Target channel, use the PTC (tube 1) EPF to set the threshold.
- For the Endogenous Control, use the average EPF from samples to set the threshold.

Results interpretation:

Reaction Type		qPCR Signal	
Positive control (tube 1)		18.5 ± 2	18.5 ± 2
No template control		-	-
Sample	Endogenous Control: (VIC/HEX)	≤ 28	≤ 28
	Target: (FAM)	+	-
Result		**Positive result**	**Negative result**

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INTERPRETATION OF RESULTS – CONTROLS EXPLAINED

Positive control

The PTC (tube 1) should amplify in a Cq range of 18.5 ± 2 for the target. If this Cq range is not achieved, the sample test result is invalid and should be repeated.

To achieve the most accurate quantitative result from the positive control standard curve, an efficiency between 90% to 110% is desirable. If it falls outside of this range, preparing a fresh standard curve and repeating the run may improve the efficiency value.

Please note: The positive control is a sequence representative of the target region and does not contain the organism's entire genome. The positive control does not include the Endogenous Control sequence and should not be expected to amplify in the VIC/HEX channel.

No template control

The NTC tube/well(s) should be negative, with no amplification.

Please note: Background laboratory contamination can result in a very late signal in NTC tube/well(s). If the NTC has amplification, comparison to the sample test result is necessary:

- If the NTC is ≥5 Cq later than the sample signal, it can be considered negative, and the sample test result is valid.
- If the NTC is <5 Cq later, the sample test result is invalid – the test should be repeated after potential sources of contamination have been removed.

Endogenous Control

Detection of the Endogenous Control is through the VIC/HEX channel. The Cq value from the Endogenous Control will vary according to the amount of DNA in the sample. Cq values ≤ 28 indicate a successful extraction has taken place with sufficient host derived DNA in the sample. If the signal is later than this, repeating the nucleic acid extraction is advised.

Please note: If the sample delivers a strong positive result for the target of interest, then amplification of the Endogenous Control may be affected and may appear later. In this case, lack of Endogenous Control amplification is acceptable, and the sample test result is valid.

PRODUCT SPECIFICATIONS

Storage

Store at -20°C from arrival. The product's shelf life is outlined as an expiry date on the pouch label.

Suitable input material

This qPCR test will work well with any source of good quality nucleic acid. Good quality is defined as nucleic acid with high integrity (not degraded). Poor quality input nucleic acid is a leading cause of test failure.

Regulatory status

This product has been developed for Research Use Only (RUO) and is not intended for diagnostic use. It should not be used for diagnosis of disease or infection unless specifically approved by the regulatory authorities in the country of use.

Quality Control

In accordance with the YouSeq Ltd ISO EN 13485-certified Quality Management System, each lot of Cryptococcus neoformans qPCR Test Kit is tested against predetermined specifications to ensure consistent product quality. The primers/probe(s) typically demonstrate ≥95% in silico specificity to their intended target and periodically checked against newly available sequence information to maintain their detection profile.

Technical Assistance

For customer support, please contact:

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