



YOUSEQ

ASPERGILLUS NIGER qPCR TEST KIT USER GUIDE

CAT NO.: YSL-qP-IC-A.niger-100

100 reactions

With Internal extraction control and lyophilised MasterMix

VERSION 5.3

For research use only



YOUSEQ

YouSeq Ltd
8 Moorside Place
Moorside Road
Winchester
SO23 7FX
United Kingdom

+44 333 577 6697
hello@youseq.com

youseq.com












YOUSEQ

INTENDED USE

This product is a qPCR test kit for detection and quantification of *Aspergillus niger* (A.niger) DNA in good quality nucleic acid samples from a variety of sources. It is designed to be used by trained users in a suitable molecular biology laboratory environment.

KIT CONTENTS

| | Cap Colour | Volume |
|---|---|---------|
| A.niger specific primer/probe (FAM Probe) |  | 110 µl |
| Internal control primer/probe (VIC/HEX Probe) |  | 110 µl |
| DNase/RNase free water |  | 1.5 ml |
| Template resuspension buffer |  | 1.5 ml |
| ROX passive reference dye |  | 10 µl |
| Lyophilised Tetra 2X qPCR MasterMix |  | 1.1 ml* |
| MasterMix resuspension buffer |  | 1.5 ml |
| A.niger positive control template |  | 500 µl* |
| Internal extraction control DNA template |  | 500 µl* |

* Supplied lyophilised and requires resuspension before use, see resuspension step below for instructions

RESUSPENSION

Resuspend the designated kit contents with the correct reagents as per the table below. Spin or gently tap the vials to ensure all the contents is at the bottom before opening.

After adding the resuspension reagent, pulse vortex the vials to ensure each is mixed well.

| | Reagent | Volume |
|--|-------------------------|--------|
| Lyophilised Tetra 2X qPCR MasterMix | MasterMix resus. buffer | 1.1 ml |
| A.niger positive control template | Template resus. buffer | 500 µl |
| Internal extraction control DNA template | Template resus. buffer | 500 µl |



YOUSEQ

ROX (PLATFORM DEPENDENT)

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

If ROX is required, dilute the ROX supplied according to the table below, then add 5µl to the fully resuspended Tetra MasterMix.

| | Instruments | Step 1: Volume of water to add to ROX tube | Step 2: Add to MasterMix vial |
|-----------------------|--|--|-------------------------------|
| High ROX Instruments: | Applied Biosystems 7700, 7000, 7900, 7300, StepOne, StepOne Plus, and Roche capillary Lightcyclers 2.0 | No Dilution Required | 5 µl |
| Mid ROX Instruments: | Stratagene MX | 75 µl | 5 µl |
| Low ROX Instruments: | Applied Biosystems 7500 Platform, ViiA7 platforms, Quantstudio | 130 µl | 5 µl |
| All Other Machines | | Not Required | Not Required |

MATERIALS REQUIRED BUT NOT PROVIDED

DNA Extraction kit - This qPCR test kit will work well with high quality DNA derived from any extraction kit with minimal PCR inhibitors present.

qPCR instrument with minimum 2 colour detection (FAM and VIC/HEX).

Pipettes, micro centrifuge tubes and general laboratory equipment.

KIT SPECIFICITY

The YouSeq qPCR test kit for detection and quantification of *Aspergillus niger* (A.niger) is designed to have the broadest detection profile possible and detect all clinically relevant strains. The primers and probes have very high (>95%) homology with all reference data within the NCBI database.

The target genome region (COX1 gene) has been demonstrated to have a unique sequence in this species making it an ideal target for highly specific detection of *Aspergillus niger*.

If you require more specific data about the detection profile of the kit, please do not hesitate to contact our bioinformatics team : support@youseq.com



YOUSEQ

USE OF DNA INTERNAL CONTROL

Add 5 μ l of the internal extraction control DNA into the extraction/lysis buffer for each sample that you are extracting. Make sure not to add this DNA directly in to your biological sample as this may cause degradation of the control DNA.

Continue DNA extraction as per the manufacturer's method.

qPCR BENCH SIDE PROTOCOL

Clean and decontaminate all work surfaces, pipettes and other equipment prior to use to remove potentially contaminating nucleic acids.

REACTION SET UP

Combine the following reagents to create a final test reaction:

| Component | Volume |
|--|------------|
| Tetra 2X qPCR MasterMix | 10 μ l |
| A.niger specific primer/probe | 1 μ l |
| Internal extraction control primer/probe | 1 μ l |
| Extracted Sample DNA | 8 μ l |
| Final Volume | 20 μ l |

NEGATIVE CONTROL

For a negative control reaction, repeat the reaction set up above replacing the sample DNA with DNase/RNase free water.



Please note: Make sure to seal the sample and negative control wells before proceeding to the positive control step.



POSITIVE CONTROL

In your designated post-PCR environment, perform a serial dilution of the positive control template to create a six-point standard curve.

1. Add 90 μ l of template resuspension buffer into 5 tubes and label them 2,3,4,5 and 6.
2. Pipette 10 μ l of Positive Control Template into tube 2
3. Mix by pipetting up and down 5 times
4. Change pipette tip and pipette 10 μ l from tube 2 into tube 3
5. Mix by pipetting up and down 5 times

Repeat steps 4 and 5 to complete the dilution process.

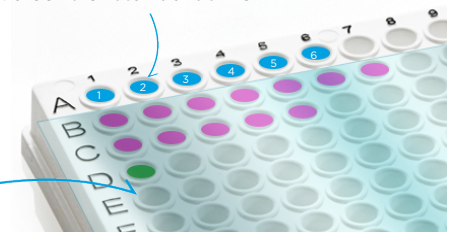
Combine the following reagents for each point of the standard curve:

| Component | Volume |
|---|------------|
| Tetra 2X qPCR MasterMix | 10 μ l |
| A.niger specific primer/probe | 1 μ l |
| Internal Control primer/probe | 1 μ l |
| Designated dilution of Positive Control | 5 μ l |
| DNase/RNase Free Water | 3 μ l |
| Final Volume | 20 μ l |

The described standard curve provides a dynamic range as in the table below:

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

Positive control standards 1-6



Other wells sealed to avoid contamination



YOUSEQ

qPCR AMPLIFICATION PROTOCOL

This YouSeq kit will work with any qPCR instrument capable of detecting FAM and VIC/HEX.

Run the following PCR protocol:

| | Temperature | Time |
|-----------|-------------|------------|
| Hot Start | 95°C | 3 minutes |
| 45 cycles | 95°C | 15 seconds |
| | 60°C* | 60 seconds |

*Make sure to collect fluorogenic data through both the FAM and VIC/HEX channels during this step

INTERPRETATION OF RESULTS

Positive control

Firstly, check the positive control performance. The undiluted positive control should amplify in a Cq range of approximately 18.5 +/-2. If the Cq range is not achieved, this would be a failed test and should be repeated.

If running a positive control standard curve for a quantitative result, then an efficiency between 90% to 110% must be achieved. This will be automatically calculated by your analysis software. If it falls outside of this range, the run should be repeated with a freshly prepared standard curve.

Negative control

In ideal circumstances, the negative control should deliver a flat line - negative result. However, it is not uncommon for background laboratory contamination to cause a very late signal. If this signal is ≥ 5 Cq values later than your sample signal then it can be considered negative and the result is viable.

If the negative control is < 5 Cq later than the signal sample, then the result is inconclusive and the test should be repeated after potential sources of contamination have been removed.

The test is valid if the following conditions are met:

| | FAM Channel |
|---------------------------|-------------|
| Positive Control (Tube 1) | + |
| Negative Control | - |



YOUSEQ

INTERPRETATION OF RESULTS CONTINUED

Positive samples

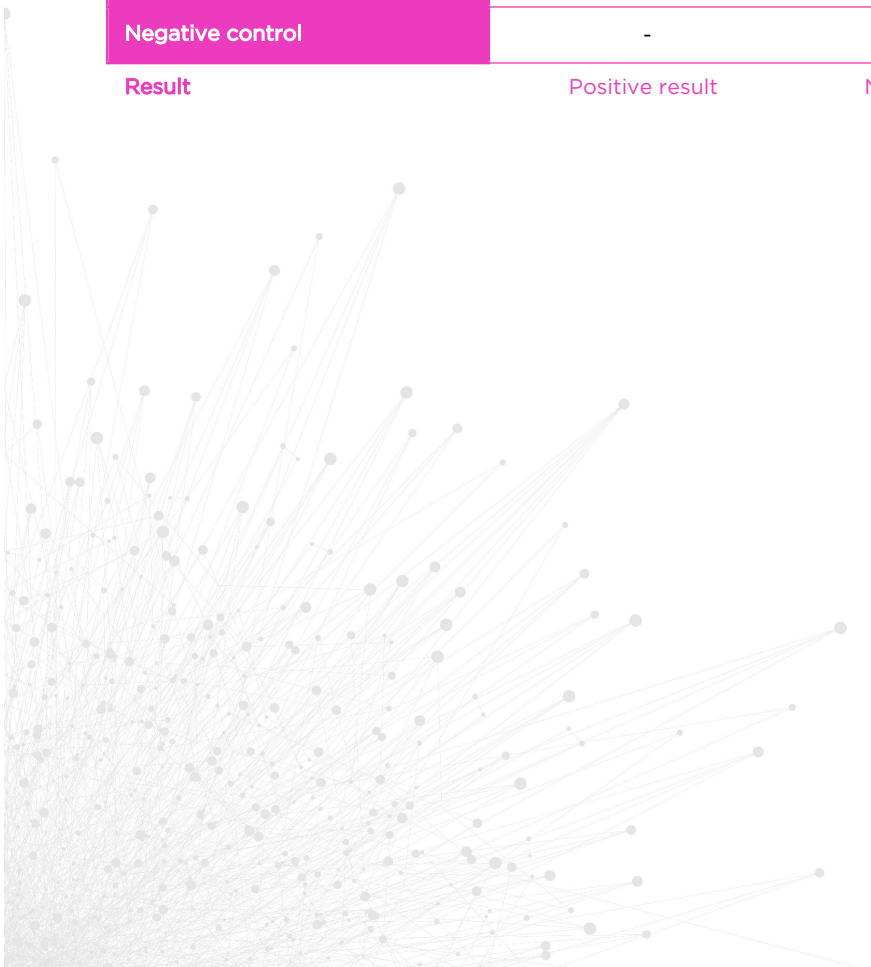
Samples that are positive for *Aspergillus niger* will deliver a defined "sigmoidal" amplification plot. Your qPCR instrument software will calculate a quantitative result for these signals by comparing the signal to the positive control standard curve. Results will be presented as target copy number.

Internal extraction control

Detection of the internal extraction control is through the VIC/HEX channel. This control gives information about the efficiency of your DNA extraction step. Cq values in the range 28±3 indicate a good quality extraction has taken place. If your signal is later than this, you may wish to consider repeating the DNA extraction.

Results interpretation at a glance:

| Sample | qPCR Signal | | |
|-----------------------------|-----------------|-----------------|-------------------------------|
| | ≤35 | - | - |
| Internal extraction control | +/- | + | - |
| Positive control | + | + | + |
| Negative control | - | - | - |
| Result | Positive result | Negative result | Failed test. Insufficient DNA |





YOUSEQ

PRODUCT SPECIFICATIONS

How sensitive is my kit?

The kit is suitable for the detection of *Aspergillus niger* (A.niger), across a wide dynamic range from 10^6 copies to less than 100 copies of the target in the PCR reaction.

Storing your kit

Store at -20°C from arrival. The qPCR kits shelf life is outlined as an expiry date on the pouch label. Once you have prepared the positive control standard curve it can be stored frozen. However, if you observe a shift in C_q values in the standard curve over time a fresh standard curve should be prepared.

Use good quality DNA

Poor quality input nucleic acid is the biggest cause of test failure. The kit will work well with any source of good quality DNA. Good quality is defined as DNA with high integrity (not degraded) and with low levels of inhibitors present.

Regulatory status

This product has been developed for Research Use Only and is not intended for diagnostic use. It should not be used for diagnosis of disease 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 *Aspergillus niger* qPCR Test Kit is tested against predetermined specifications to ensure consistent product quality.

Technical Assistance

For customer support, please contact:

e-mail: support@youseq.com

phone: +44 (0)333 577 6697

Trademarks and Disclaimers

YouSeq®, ABI Prism® (Applied Biosystems); CFX96™ (Bio-Rad); Rotor-Gene®, QIAamp®, QIASymphony® (QIAGEN); LightCycler® (Roche); FAM™, ROX™ (Thermo Fisher Scientific).

Registered names, trademarks, etc. used in this document, even if not specifically marked as such, are not to be considered unprotected by law.

Not available in all countries.

© 2022 YouSeq Ltd; all rights reserved.

