Primerdesign

Z-Path-Aspergillus_SCRN-V2.0

# Aspergillus

Selective screening kit

## Kit version: 2

## Target regions:

Aspergillus flavus (mdr1 gene)

Aspergillus fumigatus (cyp51A gene)

Aspergillus niger (SAR1 gene)

Aspergillus terreus (gcd gene)

genesig® kit

150 tests

GENESIG

Kits by Primerdesign

For general laboratory and research use only

# Product Description

This genesig® Aspergillus selective screening kit detects four species of Aspergillus (A. flavus, A. fumigatus, A. niger and A. terreus) but does not differentiate between them. These species can each cause aspergillosis, with symptoms that can range from mild to life-threatening.

# Specificity

The kit is designed for the in vitro detection of A. flavus, A. fumigatus, A. niger and A. terreus with a broad detection profile for these pathogens. Specifically, the primers will detect over 95% of sequences available on the NCBI database at the time of last review.

The A. flavus assay is predicted to cross-react with A. parasiticus and A. oryzae.

The dynamics of genetic variation mean that new sequence information may become available after the most recent review. If you require further information or have a specific question about the detection profile of this kit, then please send an email to techsupport@primerdesign.co.uk and our team will answer your question.

# Kit contents

| Quantity | Component | Tube | Cap Colour |
|---|---|---|---|
| 1 | **Aspergillus_SCRN-v2.0 primer/probe mix (including IEC primer/probe mix)**<br>**(150 reactions)**<br>FAM labelled, target: A. flavus<br>FAM labelled, target: A. fumigatus<br>FAM labelled, target: A. niger<br>FAM labelled, target: A. terreus<br>VIC labelled, target: Internal extraction control |  | **BROWN** |
| 1 | **Aspergillus_SCRN-v2.0 positive control template** |  | **RED** |
| 1 | **Internal extraction control DNA** |  | **BLUE** |
| 1 | **Template preparation buffer**<br>For resuspension of the positive control template and Internal extraction control DNA |  | **YELLOW** |
| 1 | **RNase/DNase free water**<br>For resuspension of the primer/probe mix |  | **WHITE** |

# Reagents and equipment to be supplied by the user

### Real-time PCR Instrument

### Extraction kit

This kit is recommended for use with genesig® Easy DNA/RNA Extraction kit or exsig®Mag. However, it is designed to work well with all processes that yield high-quality nucleic acid with minimal PCR inhibitors.

### oasig® lyophilised or PrecisionPLUS® 2X qPCR Master Mix

This kit is intended for use with oasig® lyophilised or PrecisionPLUS® 2X qPCR Master Mix.

### Pipettors and filter tips

### Vortex and centrifuge

### 1.5 ml tubes

### qPCR plates or reaction tubes

# Kit storage and stability

This kit is stable for shipping at ambient temperature but should be stored at -20°C upon arrival. Once the lyophilised components have been resuspended, they should not be exposed to temperatures above -20°C for longer than 30 minutes at a time and unnecessary repeated freeze/thawing should be avoided. The kit is stable for six months from the date of resuspension under these circumstances.

Primer Design Ltd does not recommend using the kit after the expiry date stated on the pack.

# Suitable sample material

This kit can be used with all types of samples from various origins. Please ensure that the extracted nucleic acid samples are suitable in terms of purity, concentration, and DNA integrity. An internal extraction control is provided to test for non-specific PCR inhibitors.

# Dynamic range of test

Under optimal PCR conditions the kit can achieve priming efficiencies between 90-110% and detect less than 100 copies of target template.

# Principles of the test

## Real-time PCR

A target specific primer and probe mix is provided, and this can be detected through the FAM channel.

The primer and probe mix provided exploits the so-called TaqMan® principle. During PCR amplification, forward and reverse primers hybridize to the target DNA. A fluorogenic probe is included in the same reaction mixture which consists of a DNA probe labelled with a 5′-dye and a 3′-quencher. During PCR amplification, the probe is cleaved, and the reporter dye and quencher are separated. The resulting increase in fluorescence can be detected on a range of qPCR platforms.

## Positive control

The kit contains a single positive control that contains templates for one of the Aspergillus species and will give a result through the FAM channel. Each time the kit is used, at least one positive control reaction must be included in the run. A positive result indicates that the primers and probes for detecting the target genes worked properly in that particular experimental scenario. If a negative result is obtained the test results are invalid and must be repeated. Care should be taken to ensure that the positive control does not contaminate any other kit component which would lead to false-positive results. This can be achieved by handling this component in a post-PCR environment. Care should also be taken to avoid cross-contamination of other samples when adding the positive control to the run. This can be avoided by sealing all other samples and negative controls before pipetting the positive control into the positive control well.

## Negative control

To validate any positive findings a negative control reaction should be included every time the kit is used. For this reaction, the RNase/DNase free water should be used instead of template. A negative result indicates that the reagents have not become contaminated while setting up the run. It is also known as a No Template Control or NTC.

## Internal DNA extraction control

When performing DNA extraction, it is often advantageous to have an exogenous source of DNA template that is spiked into the lysis buffer. This control DNA is then co-purified with the sample DNA and can be detected as a positive control for the extraction process. Successful co-purification and qPCR for the control DNA also indicates that PCR inhibitors are not present at a high concentration.

Within the multiplex primer/probe mix are primers and probes to detect the exogenous DNA using qPCR. The primers are present at PCR limiting concentrations which allows multiplexing with the target sequence primers. Amplification of the control DNA does not interfere with detection of the target DNA even when present at low copy number. The Internal control is detected through the VIC channel and gives a Cq value of 28+/-3.

# Resuspension protocol

To minimise the risk of contamination with foreign DNA, we recommend that all pipetting is performed in a PCR clean environment. Ideally this would be a designated PCR lab or PCR cabinet. Filter tips are recommended for all pipetting steps.

1. **Pulse-spin each tube in a centrifuge before opening.**
   This will ensure lyophilised primer/probe mix is in the base of the tube and is not lost upon opening the tube.

2. **Resuspend the primer/probe mix in the RNase/DNase free water supplied, according to the table below:**
   To ensure complete resuspension, allow primer/probe mix to rehydrate for 10 minutes at room temperature. Vortex the tube thoroughly, followed by pipetting up and down 10 times. Failure to mix well can produce poor kit performance.

| Component – resuspend in water | Volume |
|---|---|
| **Pre-PCR pack** | |
| Aspergillus_SCRN-v2.0 primer/probe mix (**BROWN**) | 165µl |

3. **Resuspend the positive control template in the template preparation buffer supplied, according to the table below:**
   To ensure complete resuspension, vortex the tube thoroughly.

| Component – resuspend in template preparation buffer | Volume |
|---|---|
| **Pre-PCR heat-sealed foil** | |
| Internal extraction control DNA (**BLUE**) | 600µl |
| **Post-PCR heat-sealed foil** | |
| Aspergillus_SCRN-v2.0 positive control template (**RED**)* | 500µl |

*** This component contains high copy number template and is a VERY significant contamination risk. It must be opened and handled in a separate laboratory environment, away from the other components.**

# DNA extraction

The internal extraction control DNA can be added to either lysis/extraction buffer or to the sample once it has been resuspended in lysis buffer.

**DO NOT add the internal extraction control DNA directly to the unprocessed biological sample as this will lead to degradation and a loss in signal.**

1. **Add 4µl of the Internal extraction control DNA (BLUE) to each sample in DNA lysis/extraction buffer per sample.**

2. **Complete DNA extraction according to the manufacturer's recommended protocols.**

# qPCR detection protocol

**For optimum performance and sensitivity**
All pipetting steps and experimental plate set up should be performed on ice. After the plate is prepared, proceed immediately to the amplification protocol. Prolonged incubation of reaction mixes at room temperature can lead to PCR artifacts that reduce the sensitivity of detection.

1. **For each DNA sample prepare a reaction mix according to the table below:**
   Include sufficient reactions for positive and negative controls.

| Component | Volume |
|---|---|
| oasig® lyophilised or PrecisionPLUS® 2X qPCR Master Mix | 10µl |
| Aspergillus_SCRN-v2.0 primer/probe mix (**BROWN**) | 1µl |
| RNase/DNase free water (WHITE) | 4µl |
| **Final volume** | **15µl** |

2. **Pipette 15µl of this mix into each well according to your qPCR experimental plate set up.**

3. **Pipette 5µl of DNA sample into each well according to your experimental plate set up.**
   For negative control wells use 5µl of RNase/DNase free water (WHITE). The final volume in each well is 20µl.

4. **Pipette 5µl of positive control template into each well according to your plate set up.**
   The positive control (**RED**) contains template for Aspergillus flavus. The final volume in each well is 20µl.

# qPCR amplification protocol

Amplification conditions using oasig® lyophilised or PrecisionPLUS® 2X qPCR Master Mix.

| | Step | Time | Temp |
|---|---|---|---|
| Cycling x50 | Enzyme activation | 2 min | 95 ℃ |
| | Denaturation | 10 s | 95 ℃ |
| | DATA COLLECTION * | 60 s | 60 ℃ |

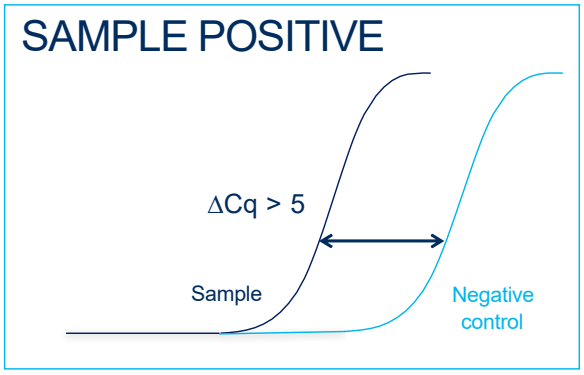
* Fluorogenic data should be collected during this step through the FAM and VIC channels.

# Interpretation of results

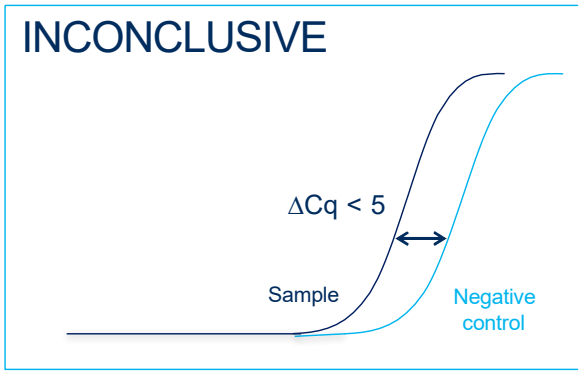
| Target (FAM) | Internal extraction control (VIC) | Positive Control | Negative Control | Interpretation |
|---|---|---|---|---|
| + | + / - | + | - | **ASPERGILLUS POSITIVE RESULT** |
| - | + | + | - | **NEGATIVE RESULT** |
| + / - | + / - | + | ≤35 | **EXPERIMENT FAILED Due to test contamination** |
| + / - | + / - | + | >35 | * |
| - | - | + | - | **SAMPLE PREPARATION FAILED** |
| + / - | + / - | - | + / - | **EXPERIMENT FAILED** |

Positive control template (**RED**) is expected to amplify between Cq 16 and 23. Failure to satisfy this quality control criterion is a strong indication that the experiment has been compromised.

* Where the test sample is positive, and the negative control is positive with a Cq >35, the sample must be reinterpreted based on the relative signal strength of the two results:




If the sample amplifies > 5 Cq earlier than the negative control, then the sample should be reinterpreted (via the table above) with the negative control verified as negative.




If the sample amplifies < 5 Cq earlier than the negative control, then the positive sample result is invalidated, and the result should be determined inconclusive due to test contamination. The test for this sample should be repeated.

### Positive control

The positive control well should give an amplification plot through the FAM channel. The positive control signals indicate that the kit is working correctly. If a negative result is obtained in the FAM channel, then the test results are invalid and must be repeated.

### No template control (NTC)

The NTC should give a flat line (flat amplification plots) through all channels. Signals in the NTC indicate cross contamination during plate set up.

### Internal DNA extraction control

The Cq value obtained with the internal control will vary significantly depending on the extraction efficiency, the quality and quantity of DNA added to the PCR reaction and the individual machine settings. Cq values of 28±3 are within the normal range. When amplifying a target sample with a high genome copy number, the internal extraction control may not produce an amplification plot. This does not invalidate the test and should be interpreted as a positive experimental result.

### Sample data

Presence of Aspergillus species is detected in the FAM channel as indicated in the kit contents section. A positive signal in the FAM channel therefore indicates the presence of any of the four Aspergillus species detected by this kit. Mixed infections, where more than one species is present in the same sample, may occur. In these situations, detection of mixed infections can lead to abnormal shaped curves, but these can still be interpreted as detailed above.

# Notices and disclaimers

This product is developed, designed and sold for research purposes only. It is not intended for human diagnostic or drug purposes or to be administered to humans unless clearly expressed for that purpose by the Food and Drug Administration in the USA or the appropriate regulatory authorities in the country of use. During the warranty period, Primer Design Ltd genesig® detection kits allow precise and reproducible data recovery combined with excellent sensitivity. For data obtained by violation of the general GLP guidelines and the manufacturer's recommendations the right to claim under guarantee is expired.

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