Primerdesign™ Ltd

# Aspergillus

## Selective screening kit

## genesig® kit

150 tests

GENESIG

Kits by Primerdesign

For general laboratory and research use only

# Introduction to *Aspergillus*

This kit detects four species of *Aspergillus*; *aspergillus fumigatus*, *aspergillus niger*, *aspergillus terreus* and *aspergillus flavus*.

*Aspergillus fumigatus* is a pathogenic fungus of the genus *Aspergillus*. It is a saprotroph that is found widely in nature, typically in soil and decaying organic matter, such as compost heaps, and is the causative agent for the disease aspergillosis. A. fumigatus has a haploid 29.4 Mb genome that forms eight chromosomes. It forms colonies that produce hyphae known as conidiophores that produce thousands of minute grey-green conidia (2–3 µm) that readily become airborne. Inhaling the spores can result in allergic bronchopulmonary aspergillosis, pulmonary aspergilloma or invasive aspergillosis. Infection occurs after inhalation and it is estimated that everybody inhales several hundred spores each day; typically, these are quickly eliminated by the immune system in healthy individuals. The fungus is more likely to become pathogenic in immunocompromised individuals.

*Aspergillus terreus* is a filamentous ascomycete fungus with a global distribution, commonly found in soil, compost and dust. It is less common that other species of the *Aspergillus* genus (e.g. *A.fumigatus*) however is often the cause of opportunistic aspergillosis. *A.terreus* was originally thought to be asexual however it is now known to be capable of sexual reproduction. The *A.terreus* genome is approximately 35 Mbp in size and has a 50-60% GC content. *A.terreus* has roughly 10,000 protein-coding genes, most of which contain multiple small introns. *A.terreus* produces characteristic *Aspergillus* aerial hyphae as well as lateral cells (aleurospores) without conidiophore structures in submerged culture. Invasive aspergillosis as a result of A.terreus infection causes significantly higher mortality than infection with other Aspergillus species.

*Aspergillus niger* is one of the most common species of the *Aspergillus* genus. *A.niger* is a common contaminant of food causing a disease called black mould on certain fruits and vegetables including grapes, apricots, peanuts and onions. As with other species of *Aspergillus*, *A.niger* is ubiquitous in the soil. *A.niger* can cause aspergillosis however infections in humans are generally very rare.

*Aspergillus flavus* is a common contaminant of cereal grains, tree nuts and legumes. Infections can occur pre-harvest however often the plant shows no symptoms until post-harvest storage and/or during transport. Many strains produce significant quantities of toxic compounds known as mycotoxins, which are toxic to mammals when consumed. Like many other species of *Aspergillus*, *A. flavus* can cause Aspergillosis in immunocompromised individuals.

# Specificity

The kit contains individual primers directed against the four following *aspergillus* species; *aspergillus fumigatus, aspergillus niger, aspergillus terreus* and *aspergillus flavus*.

The primers and probe sequences in this kit have 100% homology with a broad range of clinically relevant reference sequences based on a comprehensive bioinformatics analysis. Please note, this assay may also detect strains of *aspergillus oryzae*.

If you require further information or have a specific question about the detection profile of this kit, then please send an email to enquiry@primerdesign.co.uk and our bioinformatics team will answer your question.

# Kit contents

- **Aspergillus_SCRN primer/probe mix (150 reactions BROWN)**
  FAM and VIC labelled (see table below)

| Target | Fluorophore |
|---|---|
| ***Aspergillus fumigatus*** | FAM |
| ***Aspergillus niger*** | FAM |
| ***Aspergillus terreus*** | FAM |
| ***Aspergillus flavus*** | FAM |
| **Internal extraction control** | VIC |

- **Aspergillus_SCRN positive control template (RED)**
  Positive signal for FAM channel
- **Internal extraction control DNA (150 reactions BLUE)**
- **RNase/DNase free water (WHITE)**
  for resuspension of primer/probe mix
- **Template preparation buffer (YELLOW)**
  for resuspension of positive control template and internal control template

# Reagents and equipment to be supplied by the user

**Real-Time PCR Instrument**
Ability to detect FAM and VIC channels

**DNA extraction kit**
This kit is recommended for use with genesig Easy DNA/RNA extraction kit. However, it is designed to work well with all processes that yield high quality DNA with minimal PCR inhibitors.

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

**Pipettors and Tips**

**Vortex and Centrifuge**

**Thin walled 1.5ml PCR reaction tubes**

# Kit storage and stability

This kit is stable at room temperature but should be stored at -20ºC on arrival. Once the lyophilised components have been resuspended they should not be exposed to temperatures above -20ºC 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.
Primerdesign does not recommend using the kit after the expiry date stated on the pack.

# Suitable sample material

All kinds of sample material suited for PCR amplification can be used. Please ensure the samples are suitable in terms of purity, concentration, and RNA/DNA integrity. Always run at least one negative control with the samples. To prepare a negative control, replace the template DNA sample with RNase/DNase free water.

# Dynamic range of test

Under optimal PCR conditions genesig kits have very high priming efficiencies of >90% and can detect between $1x10^8$ and $1x10^2$ copies of target template.

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# Principles of the test

## Real-time PCR

Individual primers and probes designed for each aspergillus species have been combined into a single reaction and these can be detected through the FAM channel as described in the kit contents.

The primer/probe mix provided exploits the so-called TaqMan® principle. During PCR amplification, forward and reverse primers hybridise to the target DNA. Fluorogenic probes are included in the same reaction mixture, which consists of a DNA probe labelled with a 5`-dye and a 3`-quencher. During qPCR 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

For a positive control, the kit contains a single positive control that contains template 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 primer/probe mix and master mix are working properly in that run. If a negative result is obtained the test results are invalid and must be repeated. Care should be taken to ensure that the positive controls do not contaminate any other kit component, which would lead to false positive results. This can be achieved by handling these components 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 confirm the absence of contamination, 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.

## 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. The Aspergillus_SCRN primer/probe mix supplied already contains separate primers and probe 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 Aspergillus 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 be 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 spilt upon opening the tube.

**2. Resuspend the Aspergillus_SCRN primer/probe mix in the RNase/DNase free water supplied, according to the table below:**
To ensure complete resuspension, vortex the tube thoroughly.

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

**3. Resuspend internal control template and positive control template and in the template resuspension 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**) | 600 µl |
| **Post-PCR heat-sealed foil** | |
| Positive control template (**RED**)* | 500 µl |

*** 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 either to the DNA lysis/extraction buffer or to the DNA 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 protocols.

# qPCR detection protocol

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 or PrecisionPLUS 2X qPCR Master Mix | 10 µl |
| Aspergillus_SCRN primer/probe mix (**BROWN**) | 1 µl |
| RNase/DNase free water (WHITE) | 4 µl |
| **Final volume** | **15 µl** |

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

3. **Prepare sample DNA templates for each of your samples.**

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

5. **Pipette 5µl of positive control template into the positive control wells.**

# qPCR amplification protocol

Amplification conditions using oasig or PrecisionPLUS 2X qPCR Master Mix

| | Step | Time | Temp |
|---|---|---|---|
| | Enzyme activation | 2 mins | 95°C |
| X 50 cycles | Denaturation | 10 secs | 95°C |
| | **DATA COLLECTION*** | 60 secs | 60°C |

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

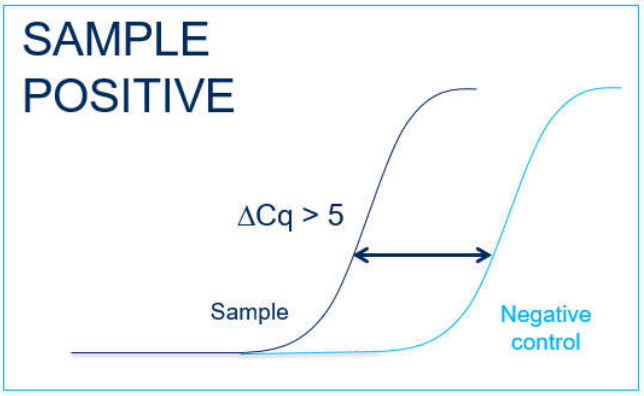
# Interpretation of results

| Target (FAM) | Internal control (VIC) | Positive Control | Negative Control | Interpretation |
|---|---|---|---|---|
| + | + / - | + | - | **POSITIVE QUALITATIVE RESULT** |
| - | + | + | - | **NEGATIVE RESULT** |

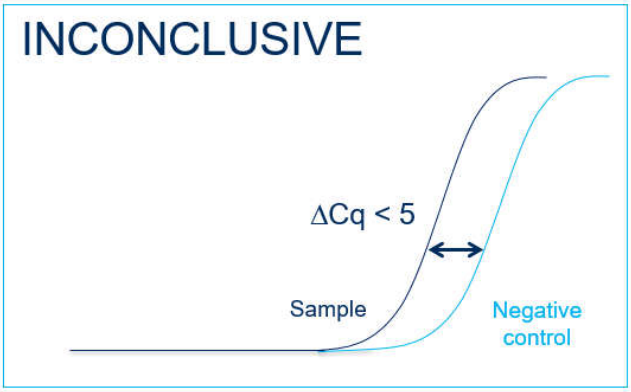
| | | | | |
|---|---|---|---|---|
| + / - | + / - | + | ≤35 | **EXPERIMENT FAILED**<br>Due to test contamination |
| + / - | + / - | + | >35 | * |
| - | - | + | - | **SAMPLE PREPARATION FAILED** |
| + / - | + / - | - | + / - | **EXPERIMENT FAILED** |

Positive control template (**RED**) is expected to amplify between Cq 16 and 23. Failure to satisfy these quality control criteria 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.

**Internal PCR control**

The Cq value obtained with the internal control will vary significantly depending on the extraction efficiency, the 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 an Aspergillus_SCRN 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.

**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). Signals in the NTC indicate cross contamination during plate set up.

**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, this kit does not allow you to determine which specific species are present in your samples. Mixed infections, where more than one species are present in the same sample, may occur. In these situations, detection of mixed infections can lead to unusually shaped curves however this is normal, and the data should not be interpreted any differently.