

MycoReal[®] Kit *Aspergillus*

Kit version 1.1



For *in vitro* diagnostic use only

MycoReal[®] Kit *Aspergillus*

Order no.	Reactions	Pathogen	Internal positive control
DHUF00253	50	FAM + VIC channel	Cy5 channel

Kit contents:

- Detection Assay 1 for *A. flavus* (FAM), *A. fumigatus* (VIC) and of internal DNA positive control (IPC) (Cy5)
- Detection Assay 2 for *A. nidulans*, *A. niger* (FAM), *A. terreus* (VIC) and of internal DNA positive control (IPC) (Cy5)
- IPC-Target DNA (control of DNA extraction and of PCR amplification)
- DNA reaction mix (contains a highly purified Taq Polymerase for rapid hot-start PCR, dNTPs with dUTP and Uracil-N glycosylase (UNG) to eliminate amplicon carryover, ROX™ dye (passive reference) and buffer components – additives optimized to handle PCR inhibitors)
- Positive control for *A. fumigatus* + *A. flavus* + *A. nidulans* + *A. terreus*
- Nuclease-free water



Pathogen information: *Aspergillus* is a fungal genus consisting of several hundred species. *Aspergillus* species are ubiquitously found and some species can cause opportunistic infections such as allergic bronchopulmonary aspergillosis, pulmonary aspergilloma and invasive aspergillosis. Invasive aspergillosis is increasingly recognized as a primary cause of morbidity and mortality especially in immunocompromised patients. *Aspergillus fumigatus* is the predominant disease-causing species. However, other species than *A. fumigatus*, in particular *A. terreus* and *A. flavus* but also *A. niger*, *A. nidulans* and *A. ustus* have gained greater clinical significance.

Not only the detection, but also the identification of the fungal species is important. The species might provide information on a potential contamination, colonisation or infection and moreover antifungal resistance.

Intended purpose: MycoReal[®] Kit *Aspergillus* is a non-automated CE-certified IVD real-time PCR test for the qualitative detection of DNA (ITS2-region) of *Aspergillus fumigatus*, *Aspergillus flavus*, *Aspergillus terreus*, *Aspergillus niger* and *Aspergillus nidulans*. Furthermore, it detects some other closely related *Aspergillus/Emericella* species belonging to *Aspergillus* section *Fumigati*, *Flavi*, *Terrei*, *Nigri*, *Nidulantes*, *Usti* and *Versicolori*.

Proper specimens are DNA extracts isolated from human samples of EDTA-blood, bronchoalveolar lavage (BAL), aspirate, cerebrospinal fluid (CSF), tissue and paraffin-embedded tissue.

This test is suitable for patients with suspected infection with *Aspergillus* and is intended as an aid in the diagnosis of infection with this pathogen in combination with patient history and additional clinical information. The test is intended for professional use and is limited to qualified personnel instructed in the procedures of real-time PCR and *in vitro* diagnostic procedures.

The analysis is performed with 2 different assay mixes in two parallel PCR reactions and thus allows the differentiation of individual species, enabling targeted antifungal therapy. A probe-specific amplification-curve in the FAM or VIC channel indicates amplification of *Aspergillus* specific DNA. The Internal Positive Control (IPC) is detected in the fluorescence channel Cy5 and serves as a control for DNA extraction and possible real-time PCR inhibition. The target for the IPC (artificial target DNA) is added during sample extraction.

PCR-platforms: This test has been validated with the ABI[®] 7500 Fast instrument (Thermo Fisher Scientific, fast cycle parameters are not supported) and tested with a cobas z 480 Analyzer (Roche). The test is also compatible with other real-time PCR instruments which detect and differentiate fluorescence in FAM, VIC and Cy5 channel (e.g., QuantStudio™ 5, QuantStudio™ 7

real-time PCR system (Thermo Fisher Scientific), qTOWER³G (Analytik Jena), Mic instrument (bio molecular systems), cobas z 480 Analyzer (Roche)).

Performance data: MycoReal[®] Kit *Aspergillus* has a LoD95% (defined as the concentration, where 95% of 20 PCR repeats were positive) between 2-11 target copies/PCR, depending on the species. Information on detectable species see intended purpose.

Results of clinical validation:

Statistic	Value	95% CI
Sensitivity	90.48%	69.62% - 98.83%
Specificity	100%	75.29% - 100.00%
Prevalence	61.76%	43.56% – 77.83%
Positive predictive value (PPV)	100%	
Negative predictive value (NPV)	86.67%	63.50% - 96.05%
Accuracy	94.12%	80.32% - 99.28%

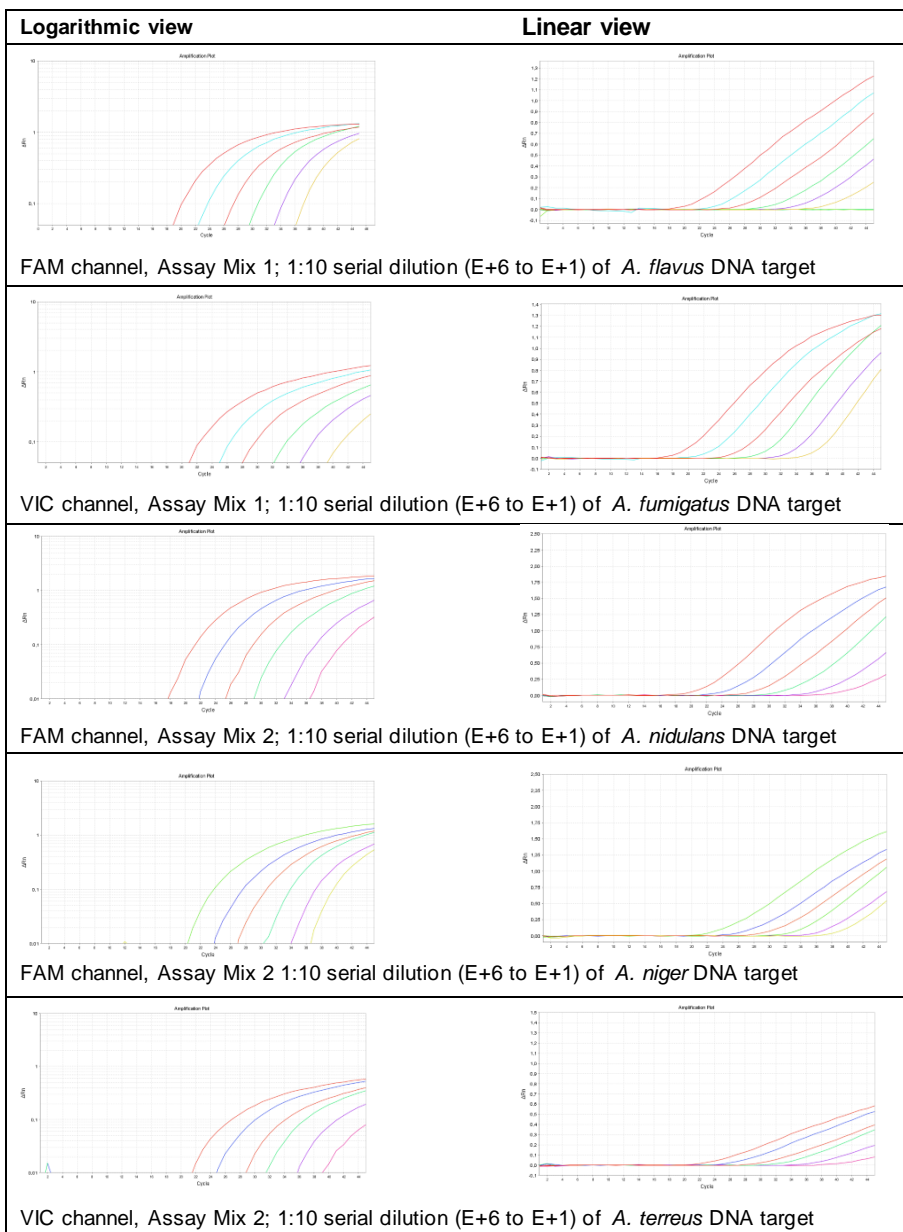


Figure 1 Performance of MycoReal[®] Kit *Aspergillus*