

# MycoReal *Aspergillus*

Order No.: RTPM100

Unit: 50 reactions



For research use only, not for diagnostic use

**Background:** *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 one most often *Aspergillus* species causing disease. 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.

**PCR-platforms:** MycoReal *Aspergillus* is developed and validated for the LightCycler® 2.0 instrument (Roche).

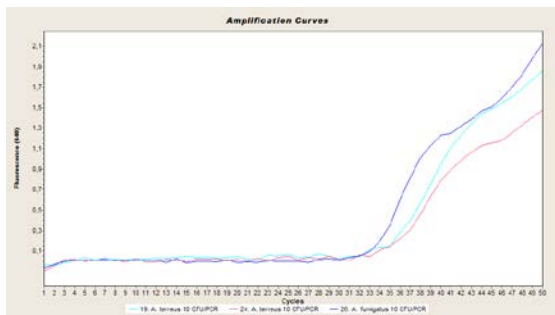
**Description:** MycoReal *Aspergillus* is based on the amplification and detection of the internal transcribed spacer 2 (ITS2-region) of *Aspergillus* using real-time PCR. It allows the rapid and sensitive detection as well as differentiation of 5 medically important species of *Aspergillus* (*A. fumigatus*, *A. flavus*, *A. nidulans*, *A. niger* and *A. terreus*). Species can be differentiated by specific melting curves. The assay is validated for DNA samples purified from bronchoalveolar lavages (BAL), blood, aspirates, cerebrospinal fluid, tissue and paraffin embedded tissue. *Aspergillus* DNA can be recovered efficiently from samples using a modified protocol (included in the manual) of the High Pure PCR Template Preparation Kit (Roche Diagnostics).

**Content:** MycoReal *Aspergillus* contains an assay with primers and species-specific probes detected at 640 nm or 705 nm for the detection and differentiation of 5 different *Aspergillus* species. Furthermore, it contains an internal positive control assay detecting an internal control target at 610 nm to exclude false-negative interpretation of results caused by inhibition of real-time PCR, two positive control samples for *A. fumigatus* and *A. niger*, a negative control sample and a manual. The amplification mix is not included. Store components at -20°C and protect from light.

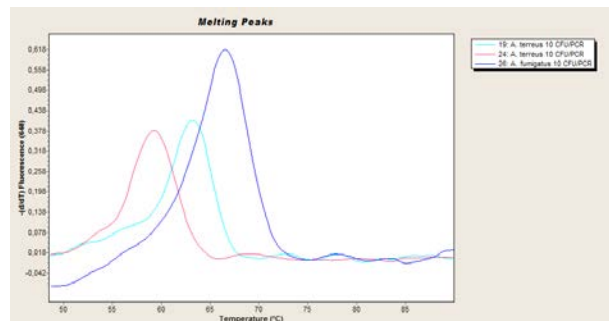
For real-time PCR amplification using the LightCycler® instrument, ingenetix recommends the use of LC™ FastStart DNA Master Kit Hybridisation Probes (Roche Diagnostics order no. 12239272001; kit for 480 reactions of 20 µl final reaction volume, or order no. 03003248001; kit for 96 reactions of 20 µl final reaction volume).

**Specificity and sensitivity:** MycoReal *Aspergillus* has a LoD 95% (defined as the concentration, where 95% of 20 PCR repeats were positive) of 3 CFU/PCR. It detects the *Aspergillus* species described above and some strains of other *Aspergillus*, *Emericella* und *Neosartorya* species. It does not show cross reactivity with other moulds. The specificity was tested on a great variety of different *Dematiaceae*, dermatophytes, moulds and yeasts.

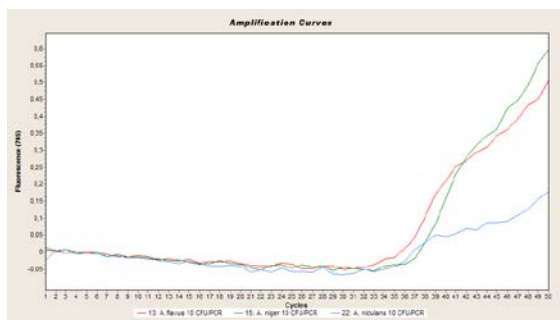
Ingenetix also offers MycoReal *Candida* (ingenetix order no. RTPM200) and MycoReal *Pneumocystis* (ingenetix order no. RTPM400, RTPM401 or RTPM403) for the detection and differentiation of 7 different *Candida* species or *Pneumocystis jiroveci*, and MycoReal Fungi (ingenetix order no. RTPM300) for the universal detection of fungal DNA. All ingenetix MycoReal assays are optimized to run under the same thermal cycling conditions and with the same amplification mix using the LightCycler® 2.0 instrument.



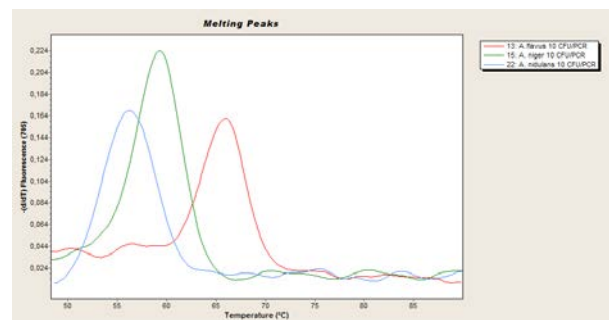
Amplification curves of 10 CFU/PCR of *A. fumigatus* and *A. terreus* (640 nm)



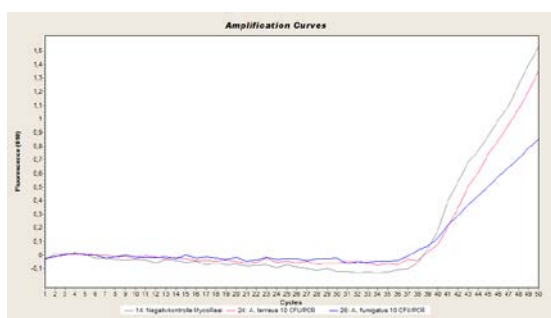
Species identification: Melting curves of 10 CFU/PCR of *A. fumigatus* (67°C) and 2 different strains of *A. terreus* (59°C or 63°C) (640 nm)



Amplification curves of 10 CFU/PCR of *A. flavus*, *A. niger* and *A. nidulans* (705 nm)



Species identification: Melting curves of 10 CFU/PCR of *A. flavus* (67°C), *A. niger* (60°C) and *A. nidulans* (56°C) (705 nm)



Amplification curves of the internal positive control (610 nm)

**References:** Schabereiter-Gurtner, C., B. Selitsch, M. Rotter, A. M. Hirschl, and B. Willinger. 2007. Development of novel real-time PCR assays for detection and differentiation of eleven medically important *Aspergillus* and *Candida* species in clinical specimens. *J. Clin. Microbiol.* 45:906-914.

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