AMD Ltd Zena Max Aspergillus Multiplex PCR Detection Kit

CE IVD

REF **KD485618-100**

Advanced Molecular Diagnostics Ltd is a diagnostics company specialising in the manufacture and supply of molecular biology instruments, reagents and consumables.

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Intended Use

This assay is a Real-Time in-vitro PCR test for the qualitative identification of Aspergillus. This kit enables the identification and differentiation of Aspergillus including *A. fumigatus, A. flavus, A. niger* and *A. terreus* species from human samples such as EDTA whole blood. This assay is based on hydrolysis probe detection and is a highly sensitive one-step qPCR kit.

Overview

Aspergillosis is a group of diseases caused by some Aspergillus fungi species such as *A. fumigatus, A. flavus, A. niger, and A terreus*. Once individuals who are immunocompromised are exposed, they experience shortness of breath, temperature, weight loss and cough. Which is more severe without treatment with antifungals and/or steroids.

Aspergillus fumigatus is an airborne fungus linked to respiratory infections, predominantly affecting immune-compromised individuals. *Aspergillus flavus* produces carcinogenic aflatoxins in crops and can lead to respiratory issues. *Aspergillus niger*, commonly found on decomposing vegetation, has industrial uses for enzyme and acid production. *Aspergillus terreus*, present in soil and indoor environments, can cause infections in susceptible individuals and is also employed for pharmaceutical purposes. Their roles range from potential health risks to industrial applications, reflecting their wide-ranging impact.

Principles of the test

The AMD Zena Max Aspergillum multiplex PCR Detection Kit is designed for the qualitative detection of *A. fumigatus, A. flavus, A. niger,* and *A terreus* by the real-time Polymerase Chain Reaction (PCR) method, which amplifies specific conserved DNA sequences, and fluorophore-labelled probes for the detection of amplified DNA. The assay consists of pairs of forward and reverse primers, and probes labelled with 5' fluorescent reporter dyes and a 3' quenchers. An internal Amplification Control (IC) containing a defined copy number of non-target DNA sequence is amplified in parallel with the sample DNA.

The point at which the fluorescence becomes detectable above the background, the quantification cycle (Cq), is proportional to the amount of target present in the sample. The lower the Cq, the greater the amount of target present. If, however, none of the Aspergillum species DNA is not present, a FAM, HEX, ROX or CY5.5 signal will not be produced. These assays are incorporated into a ready-to-use PCR master mix which utilises hot start technology, thus minimising non-specific reactions and ensuring maximum sensitivity.

An internal control is provided in order to assess the quality of the isolated DNA and the effect of any PCR inhibitors that may be present. This assay contains two primers and a Cy5 labelled probe, designed to a highly conserved region of the human genome, and a positive signal indicates that the DNA quality in the sample is acceptable for diagnostic testing. These assays are both incorporated into a ready-to-use PCR master mix which utilises hot start technology, thus minimising non-specific reactions and ensuring maximum sensitivity.

This *in vitro* diagnostic kit provides qualitative detection.

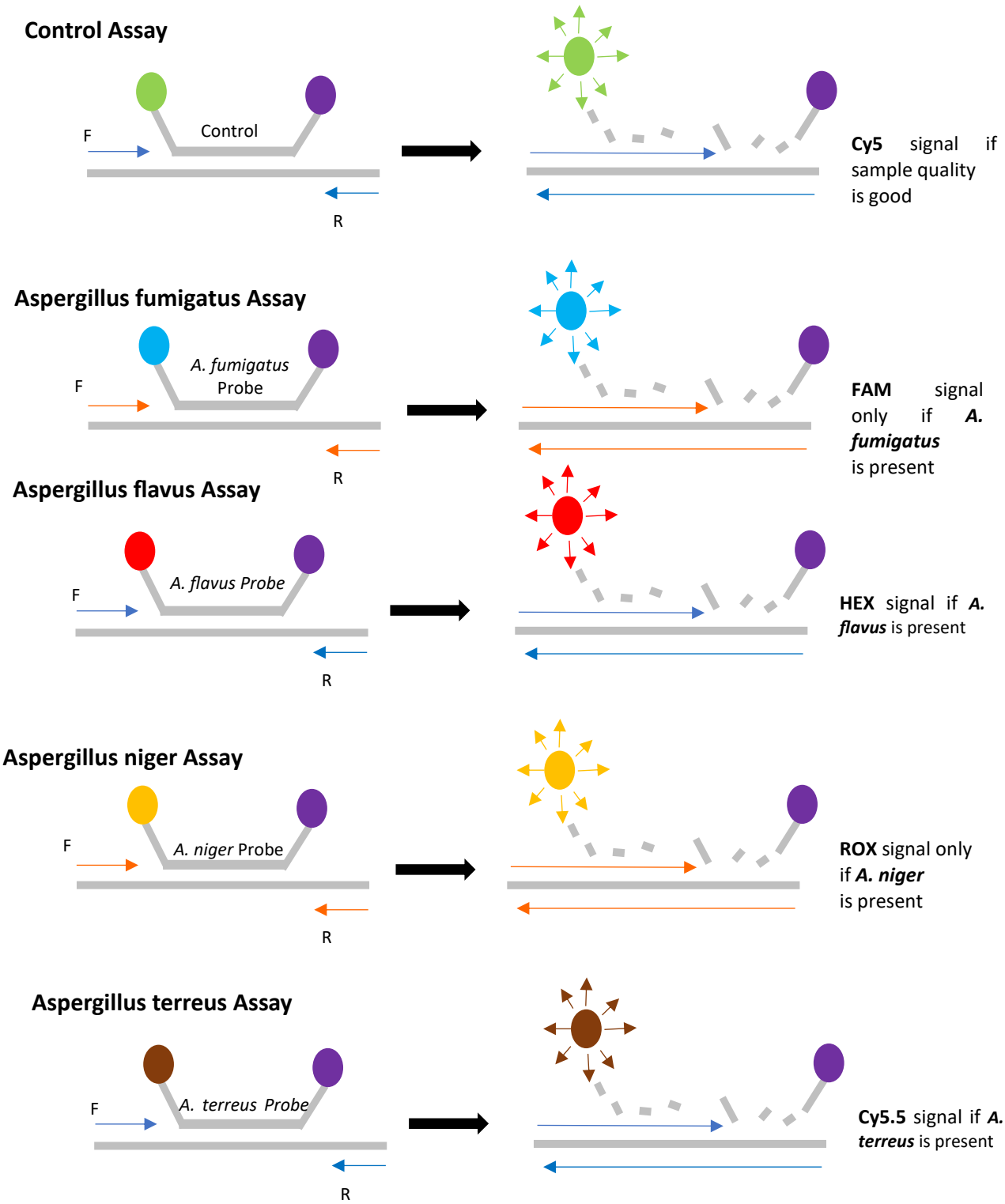

Figure 1. The principle of qPCR with hydrolysis probe detection for identifying the presence of Aspergillus DNA. The control assay in the master mix will produce a **CY5** signal if the DNA quality is acceptable. If a positive sample is tested, the Aspergillus Multiplex assay will produce the respective fluorescent signal to indicate the presence of the DNA of the specific Aspergillus species in the patient sample. If no Aspergillus

DNA from any of the four species detected in this multiplex is present, only the Cy5 signal will be detected. Due to assay competition, the Cy5 signal may be reduced or absent when other signals are strong.

Materials Provided

Kit Contents

Items	
Aspergillus qPCR M. Mix	2 x 1 ml
Aspergillus Positive Control	1 x 0.05 ml
Nuclease free water	1 x 1 ml

Reagent Storage and Handling

The kits should be transported and stored at temperatures between -15°C and -25°C and exposure to light should be avoided. If these circumstances are followed, the kit will remain stable until the expiry date printed on the package. Repeated freeze-thawing of the kit components may result in lower detection quality. To avoid that, we recommended aliquoting the master mix. Ensure that all reagents are thoroughly thawed, mixed and pulse centrifuged before use.

Materials and Equipment Required (Not Provided)

DNA Extraction: AMD recommends using the Luco DNA Extraction Kit to isolate DNA from the samples. Other leading kits or in-house methods are acceptable for use with this diagnostic kit, providing that it has been validated before use on such samples.
PCR Instrument: This kit should be used with qPCR systems which can detect FAM, HEX, ROX, Cy5.5 and Cy5 fluorescent dyes. It is also compatible with both low and high ROX instruments.
Consumables: Use nuclease-free PCR consumables appropriate to the qPCR instrument. It is also recommended that the correct PPE, including gloves, is used throughout.
Other Laboratory Equipment: Vortex, microcentrifuge, micropipettes and tips, microfuge tubes, tube rack, PCR tube/plate rack, and spectrophotometer.

Warnings and Precautions

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, please consult the appropriate safety data sheets (SDSs). Treat all samples as potentially infected. Discard sample and assay waste according to your local safety regulations. It is essential to follow the instructions in this manual precisely, to ensure accurate results. Please familiarise yourself with this product manual and your qPCR instrument before using the AMD Zena Max Aspergillus multiplex qPCR Kit.

Instrument compatibility

AMD Zena Max Aspergillus Multiplex qPCR detection kit is compatible with the most common Real-Time qPCR equipment with the capability of detecting FAM, HEX, ROX, Cy5.5 and Cy5 fluorescent dyes such as Biorad CFX96, Applied Biosystems 7500 Fast, QuantStudio 3,5,7, StepOne Plus, Aglient Mx3000, 3005P, Rotorgene Q, Cepheid Smartcycler, Analytik Jena qTower and Roche Lightcycler 480,
96.

Assay Procedure

Sample Collection

The samples to be used with this kit should be according to WHO recommendation such as EDTA whole blood samples. Please ensure that the sample is stored correctly and kept away from any contamination.

Sample Preparation

For optimal results use the Luco DNA Extraction Kit to isolate DNA from the samples. The resulting nucleic acid will be a mixture of DNA and RNA. It is important to ensure that all samples are kept free from any contamination and correct storage procedures are followed to ensure there is no damage to the DNA. Store the DNA at 2-8°C for up to 24 hours, then at -20°C for longer-term storage to ensure there is no damage to the DNA.

PCR Set up

1. Ensure that all reagents and samples are thawed completely, mixed and briefly centrifuged. Keep all reagents and samples on ice during this procedure.
2. Set up the reactions using the table below

Products	Volume x1	Volume x10
Aspergillus qPCR M. Mix	20 µl	200 µl
DNA sample/Control	5 µl	5 µl *

***Quantity per sample. Add directly to the PCR tubes/plate.**

3. Add the DNA samples to the PCR tubes/plate. Also, add 5 µl nuclease-free water in place of the DNA as a No Template Control (NTC).
4. Seal the PCR tubes or plate and briefly spin to ensure that the reagents are at the bottom and no air bubbles are present.
5. Place the plate/tubes in the qPCR thermal cycler and use the following thermal profile:

Thermal Profile:

Stage/ Step	Temperature	Time
Stage 1: Step 1	30 °C	2 min
Stage 1: Step 2	95 °C	2 min
40 Cycles		
Stage 2: Step 1	95°C	10 sec
Stage 2: Step 2	58 °C	30 sec

*Data collection step in FAM/ROX/HEX/Cy5.5 (diagnostic assay) and Cy5 (internal control assay) channels

6. When the run has finished, dispose of the PCR reaction tubes/plate appropriately under local and national regulations.

Data Analysis

Analyse the data if the software does not do this automatically at the end of the run. Export the data to Excel or a PDF report, depending on the qPCR instrument used, and view the results.

Interpretation of Results

This is a qualitative assay and the results are scored based on whether a Cq value is present or not. Results should be interpreted as follows, using the Table below as a quick reference guide.

- The internal control assay signal in the Cy5 channel should be present but may be absent or have a high Cq value (low signal) when the diagnostic assay (FAM, HEX, ROX and Cy5.5) signal is strong.
- If there is a signal in the diagnostic assay (FAM, HEX, ROX and Cy5.5) channel, with or without a Cy5 signal, the sample is **positive** for the respective Aspergillus species.
- If there is a Cy5 signal but no signal in the diagnostic channel (FAM, HEX, ROX and Cy5.5), the sample is **negative** for Aspergillus species.
- If there is no signal in either channel, the result is **inconclusive.**

Result					
CY5	**FAM**	**HEX**	**ROX**	**CY5.5**	**Interpretation**
IC	A. fumigatus	A. flavus	A. flavus	A. terreus	
Positive (Ct<34)	No Cq	No Cq	No Cq	No Cq	Negative for all species tested
No Cq	No Cq	No Cq	No Cq	No Cq	Inconclusive
Positive (Ct<34)	**Positive (Ct<38)**	No Cq	No Cq	No Cq	Positive for A. fumigatus
No Cq	**Positive (Ct<38)**	No Cq	No Cq	No Cq	Positive for A. fumigatus
Positive (Ct<34)	No Cq	**Positive (Ct<38)**	No Cq	No Cq	Positive for A. flavus
No Cq	No Cq	**Positive (Ct<38)**	No Cq	No Cq	Positive for A. flavus
Positive (Ct<34)	No Cq	No Cq	**Positive (Ct<38)**	No Cq	Positive for A. flavus
No Cq	No Cq	No Cq	**Positive (Ct<38)**	No Cq	Positive for A. flavus
Positive (Ct<34)	No Cq	No Cq	No Cq	**Positive (Ct<38)**	Positive for A. terreus
No Cq	No Cq	No Cq	No Cq	**Positive (Ct<38)**	Positive for A. terreus

Technical Specifications

Quality: All AMD kits are manufactured under high-quality standard methods and unique precision, comparable with other leading commercial Aspergillus diagnostic kits.
Sensitivity: This kit is highly sensitive under our validation methods and devices.

Specificity: The Aspergillus qPCR kit is up to 100% specific for our validation methods and devices.

Product Limitations

The kit should only be used by specifically trained laboratory personnel. The expiry date of all components must be checked before use and disposed of if expired. Occasionally mutations may arise in the genomic region targeted by the primers and probes of this assay, leading to a reduction in performance or failure of the assay. The assay design and efficacy are reviewed periodically.

Additional Information

AMD produces real-time PCR kits with a wide range of applications for researchers from gene expression analysis, cDNA and population genotyping studies to the multiplex detection of several disease targets real-time PCR with excellent sensitivity and specificity.

Contact Us

If you have any queries, comments or complaints please refer to our website at:

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References

Mousavi B, Hedayati MT, Hedayati N, Ilkit M, Syedmousavi S. *Aspergillus* species in indoor environments and their possible occupational and public health hazards. Curr Med Mycol. 2016 Mar;2(1):36-42. doi: 10.18869/acadpub.cmm.2.1.36. PMID: 28681011; PMCID: PMC5490296.

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Harmonised Symbols

The following is a key to all harmonised symbols used by AMD Ltd (Advanced Molecular Diagnostics) in Instructions for Use (IFUs) and product labelling.

Symbol	Definition	Details
	Manufacturer name and address	AMD Ltd BioCity Nottingham, Pennyfoot Street, Nottingham NG1 1GF United Kingdom
	UDI-DI number for the product given	Basic: 506105998ASMPBR UDI-DI: (01)05061059980885 UDI-PI: See label
	Minimum and maximum storage temperatures for this product	-15 to -25 degrees Celsius
	Catalogue number	KD485618-100
	Number of tests/reactions in this pack	100
	CE-IVD certified	According to Directive 98/79/EC