



Human Aspergillus fumigatus IgG Antibody ELISA Kit

Human Aspergillus fumigatus IgG Antibody ELISA Kit has been designed for the qualitative determination of specific IgG antibodies against Aspergillus fumigatus in serum and plasma (Citrate, heparin).

Catalog number: ARG82864

Package: 96 wells

For research use only. Not for use in diagnostic procedures.

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INTRODUCTION

Aspergillus fumigatus is a species of fungus in the genus *Aspergillus*, and is one of the most common *Aspergillus* species to cause disease in individuals with an immunodeficiency.

Aspergillus fumigatus, a saprotroph widespread in nature, is typically found in soil and decaying organic matter, such as compost heaps, where it plays an essential role in carbon and nitrogen recycling. Colonies of the fungus produce from conidiophores; thousands of minute grey-green conidia (2–3 µm) which readily become airborne. For many years, *A. fumigatus* was thought to only reproduce asexually, as neither mating nor meiosis had ever been observed. In 2008, *A. fumigatus* was shown to possess a fully functional sexual reproductive cycle, 145 years after its original description by Fresenius. Although *A. fumigatus* occurs in areas with widely different climates and environments, it displays low genetic variation and a lack of population genetic differentiation on a global scale. Thus, the capability for sex is maintained, though little genetic variation is produced.

The fungus is capable of growth at 37 °C or 99 °F (normal human body temperature), and can grow at temperatures up to 50 °C or 122 °F, with conidia surviving at 70 °C or 158 °F—conditions it regularly encounters in self-heating compost heaps. Its spores are ubiquitous in the atmosphere, and everybody inhales an estimated several hundred spores each day; typically, these are quickly eliminated by the immune system in healthy individuals. In immunocompromised individuals, such as organ transplant recipients and people with AIDS or leukemia, the fungus is more likely to become pathogenic, over-running the host's weakened defenses and causing a range of diseases

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generally termed aspergillosis. Due to the recent increase in the use of immunosuppressants to treat human illnesses, it is estimated that *A. fumigatus* may be responsible for over 600,000 deaths annually with a mortality rate between 25 and 90%. Several virulence factors have been postulated to explain this opportunistic behaviour.

When the fermentation broth of *A. fumigatus* was screened, a number of indolic alkaloids with antimitotic properties were discovered. The compounds of interest have been of a class known as tryprostatins, with spirotryprostatin B being of special interest as an anticancer drug. [Provided by Wikipedia: *Aspergillus fumigatus*]

PRINCIPLE OF THE ASSAY

This assay employs the enzyme immunoassay technique. Specific antigen has been pre-coated onto a microtiter plate. Each sample or Control A to C are pipetted into the wells and any specific Antibody present is bound by the immobilized antigen. After washing away any unbound substances, a HRP-conjugated anti human IgG antibody is added to each well and incubate. After washing away any unbound antibody-enzyme reagent. The immune complex formed by the bound conjugate is visualized by adding TMB substrate which gives a blue reaction product. The intensity of this product is proportional to the amount of specific antibodies in the sample. The color development is stopped by the addition of Stop Solution and the intensity of the color is measured at a wavelength of 450 nm.

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MATERIALS PROVIDED & STORAGE INFORMATION

Store the unopened kit at 2-8 °C. Use the kit before expiration date.

Component	Quantity	Storage information
Antigen-coated microplate (Aspergillus fumigatus antigens)	8 X 12 strips	4°C.
Control A; blue cap; Negative Control	2 ml (Ready-to-use)	4°C.
Control B; green cap; Cut-off Control	3 ml (Ready-to-use)	
Control C; red cap; Positive Control	2 ml (Ready-to-use)	
HRP-Conjugate (Anti-human IgG); black cap	20 ml (Ready-to-use)	4°C
20X Wash buffer	50 ml	4°C
Sample Diluent Buffer	100 ml (Ready-to-use)	4°C
TMB Substrate	15 ml (Ready-to-use)	4°C (Protect from light)
STOP Solution	15 ml (Ready-to-use)	4°C
Cover foil	1 piece	4°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 450 / 620 nm
- Incubator 37°C
- Vortex / mixer
- Pipettes and pipette tips
- Deionized or distilled water
- Automated microplate washer (optional)

TECHNICAL HINTS AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Store the kit at 4°C at all times and do not use after the expiry date.
- It is very important to bring all reagents and samples to room temperature (20-25°C) and mix them before starting the test run.
- All materials of human or animal origin should be regarded and handled as potentially infectious.
- Controls are calibrated in arbitrary units against internal quality control specimens, since no international standard reference is available for this assay.
- All components of human origin used for the production of these reagents have been tested for anti-HIV antibodies, anti-HCV antibodies and HBsAg and have been found to be non-reactive.
- If crystals are observed in the 20X Wash buffer, warm up to 37°C until the crystals are completely dissolved.
- Do not interchange reagents or Microplates of different production lots.
- Ensure complete reconstitution and dilution of reagents prior to use.
- Before pipetting all reagents should be mixed thoroughly by gentle tilting or swinging. Vigorous shaking with formation of foam should be avoided.
- It is highly recommended that the standards, samples and controls be assayed in duplicates.
- Change pipette tips between the addition of different reagent or samples.
- For further internal quality control each laboratory should additionally use known samples.

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Serum: Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately. The samples can be stored at 2-8 °C up to 5 days or aliquot and store samples at ≤ -20 °C or lower for longer storage. Avoid repeated freeze-thaw cycles.

Plasma: Collect plasma using citrate or heparin as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately. The samples can be stored at 2-8 °C up to 5 days or aliquot and store samples at ≤ -20 °C or lower for longer storage. Avoid repeated freeze-thaw cycles.

Note:

- Heat inactivation of samples is not recommended.
- Before assaying, all samples should be diluted 1+100 with Sample Diluent Buffer. Dispense 10 µL of sample and 1 mL of Sample Diluent Buffer into tubes to obtain a 1+100 dilution and thoroughly mix with a Vortex.

REAGENT PREPARATION

- **1X Wash buffer:** Dilute 20X Wash buffer into distilled water to yield 1X Wash buffer. (E.g., add 50 mL of 20X Wash Buffer into 950 mL of distilled water to a final volume of 1000 mL)

ASSAY PROCEDURE

All materials should be equilibrated to room temperature (20-25°C) before use. Standards and samples should be assayed in duplicates.

1. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal it.
2. Add **100 µL** of **diluted samples** and **controls** into respective wells. Leave one well empty for the **substrate blank**.
3. Cover the plate with the foil and incubate for **60 ± 5 minutes** at **37 ± 1 °C**.
4. Aspirate each well and wash, repeating the process 2 times for a total 3 washes. Wash by filling each well with **1× Wash Buffer (300 µL)** using a squirt bottle, manifold dispenser, or autowasher. The interval between washing and aspiration should be **> 5 sec**. Complete removal of liquid at each time is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating, decanting or blotting against clean paper towels.

Note: If performing the test on ELISA automatic systems we recommend increasing the washing steps from **three** up to **five** and the volume of Washing Buffer from **300 µL** to **350 µL** to avoid washing effects.

5. Add **100 µL** of **HRP-Conjugate** into each well (**except the substrate blank well**). Incubate for **30 minutes** at **RT** in the dark.
6. Wash as according to step 4.
7. Add **100 µL** of **TMB Substrate** to each well (including the well for substrate blank). Cover the plate and incubate for exactly **15 minutes** at **RT** in the dark.

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8. Add **100 μ L** of **Stop Solution** to each well (including substrate blank wells).
9. Read the OD with a microplate reader at **450 nm** within **30 minutes**. (**620 nm** as optional reference wave length) and use the substrate controls as blank.

CALCULATION OF RESULTS

1. Adjust the ELISA Microtiterplate reader to zero using the Substrate Blank.
If - due to technical reasons - the ELISA Microplate reader cannot be adjusted to zero using the Substrate Blank, subtract its absorbance value from all other absorbance values measured in order to obtain reliable results.
2. Measure the absorbance of all wells at 450 nm and record the absorbance values for each standard / control and sample in the plate layout. Measurement using a reference wavelength of 620 nm is recommended. Where applicable calculate the mean absorbance values of all duplicates.
3. In order for an assay run to be considered valid, these Instructions for Use have to be strictly followed and the following criteria must be met:
Substrate Blank: absorbance value < 0.100
Control A (Negative control): absorbance value < 0.200 and < Cut-off
Control B (Cut-off control): absorbance value > 0.150 – 1.300
Control C (Positive control): absorbance value > Cut-off
If these criteria are not met, the test is not valid and must be repeated.

INTERPRETATION OF RESULTS

1. The Cut-off is the mean absorbance value of the Cut-off Control determinations.
2. $(\text{Sample (mean) absorbance value} \times 10) / \text{Cut-off} = U$ (Units)
3. **< 9U (Negative):** The sample contains no antibodies against the pathogen. A previous contact with the antigen (pathogen resp. vaccine) is unlikely.
4. **9-11U (Equivocal):** Antibodies against the pathogen could not be detected clearly.
It is recommended to repeat the test with a fresh sample in 2 to 4 weeks.
If the result is equivocal again the sample is judged as negative.
5. **>11U (Positive):** Antibodies against the pathogen are present. There has been a contact with the antigen (pathogen resp. vaccine).

QUALITY ASSURANCE

Cross-Reactivity

Investigation of a sample panel with antibody activities to potentially cross-reacting parameters did not reveal evidence of false-positive results due to cross-reactions.

Sensitivity

The diagnostic sensitivity is defined as the probability of the assay of scoring positive in the presence of the specific analyte. It is 100% (95% confidence interval: 83.16% - 100%).

Interferences

Interferences with hemolytic, lipemic or icteric samples are not observed up to a concentration of 10 mg/mL hemoglobin, 5 mg/mL triglycerides and 0.5 mg/mL bilirubin.

Intra-assay and Inter-assay precision

The CV value of intra-assay precision was 1.52-5.21% and inter-assay precision was 4.67-6.25%.