

Assay Performance Characteristics:

Standard range: 40-0.08ng/mL
Limit of Detection: 0.32ng/mL
Background: OD<0.1 at 450nm
Coefficient of Determination: R-squared>0.98

References:

1. Arruda, LK, Platts-Mills, TAE, Fox, JW and Chapman, MD (1990). Aspergillus fumigatus allergen I, a major IgE binding protein, is a member of the mitogillin family of cytotoxins. J. Exp. Med., 172:1529-1532.
2. Arruda LK, Platts-Mills TAE, Longbottom JL, Chapman MD (1992). Aspergillus fumigatus: Identification of 16kd, 18kd and 45kd antigens recognized by human IgG and IgE antibodies and murine monoclonal antibodies. J. Allergy Clin. Immunol. 89:1166-1176.



A list of frequently asked questions and troubleshooting guide can be found under the 'Support' tab on our web site: www.inbio.com.

www.inbio.com

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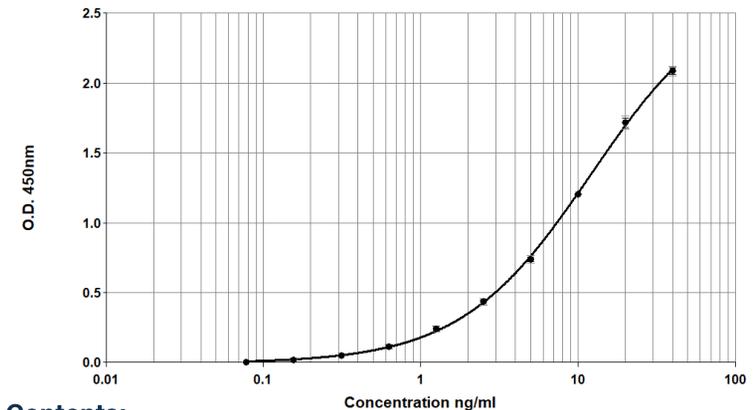
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Asp f 1 ELISA 2.0 Pre-coated Plate Kit

Product Code: EPC-AF1-X
Lot Number: xxxxx

Sample curve:



Contents:

Microtiter plate coated with anti-Asp f 1 monoclonal antibody 4A6
Asp f 1 allergen standard (white cap)
Rabbit anti-Asp f 1 polyclonal antiserum (brown cap)
Peroxidase-conjugated goat anti-rabbit IgG (blue cap)
Wash buffer (10x concentrate)
Assay buffer (10x concentrate)
TMB developing substrate
Stop solution (0.5N sulfuric acid)

Store kit at 2-8°C

Expiry:

For research and commercial use *in vitro*:
not for human *in vivo* or therapeutic use.

An InBio® product. Made in the USA.

Certificate of Analysis

Pre-coated Plate: 96-well polystyrene microtiter plate coated with monoclonal antibody 4A6 and treated with stabilizing agent. Sealed in foil pouch with desiccant.

Monoclonal Antibody: 4A6 (clone 4A6 B5 E7)
Immunogen: Asp f 1
Isotype: Mouse IgG1
Specificity: Binds to a species specific epitope on *Aspergillus fumigatus* allergen, Asp f 1.

Purification: Produced in tissue culture and purified by chromatography using Protein G. Single heavy and light chain bands on SDS-PAGE.

Lot Number: xxxxx

Detection Antibody: Polyclonal antibody raised against partially purified Asp f 1 (1,2)

Specificity: The pAb contains IgG Ab to Asp f 1 as well as IgG Ab to other *Aspergillus* allergens.

Activity: The pAb is in phosphate buffered saline, pH 7.4, containing 1% BSA/50% glycerol/PBS. The pAb has been filtered and should be diluted 1/1000 for use in Asp f 1 ELISA.

Lot Number: xxxxx

Allergen Standard: nAsp r 1

Composition: Naturally purified Asp r 1 prepared in 1% BSA, 50% glycerol/PBS, pH 7.4

Concentration: 400ng/mL (based on amino acid analysis)

Calibration: The Asp r 1 concentration of the purified natural Asp r 1 was determined by amino acid analysis. Asp f 1 and Asp r 1 (mitogillin) are 95% homologous (differing by 4 amino acid residues) and are antigenically and functionally indistinguishable (1).

Lot Number: xxxxx

Peroxidase-conjugated goat anti-rabbit:

Lot Number: xxxxx

Materials required, but not provided:

- Type I ultrapure water or 18.2M Ω de-ionized water
- Volumetric measuring equipment (e.g. serological pipettes, graduated cylinders)
- Clean containers for buffer and reagent preparation
- Reagent reservoirs
- Calibrated single and multi-channel micropipettes and tips
- Vortex mixer
- Plate reader capable of reading absorbance at 450nm
- Analysis software (recommended, but not required)

Data Processing:

4-parameter logistic curve fit (x-axis plotted on log scale)

Protocol

Please read the entire protocol before starting the assay

Bring all reagents to room temperature before use

1. Prepare a 1x dilution of wash and assay buffers from the 10x concentrates in clean containers using 18.2M Ω de-ionized water or Type I ultrapure water.
For one plate:
Wash buffer: add 15mL concentrate to 135mL water (150mL total volume)
Assay buffer: add 3mL concentrate to 27mL water (30mL total volume)
Adjust volumes accordingly for multi-plate assays. Diluted buffers may be stored at 4°C for up to 1 week.

The example below is for testing 6 samples starting at 1/10 dilution. A multichannel pipet is recommended for mixing and transferring between wells.
 - *Highly concentrated samples will require pre-dilution before adding to the plate.*
2. Remove the plate from the foil pouch. Add 150 μ L wash buffer to each well. Empty the wells by inverting the plate and then tap on absorbent paper to remove residual buffer. Repeat the wash cycle two times.
*Move directly to the next step to prevent the wells from drying.
3. Add 100 μ L assay buffer to all wells. Add an additional 80 μ L of assay buffer to wells A1-H1 (the total volume of assay buffer in these wells will be 180 μ L; all other wells will have 100 μ L).
4. **Standard:** gently vortex the standard and add 20 μ L to wells A1 and B1. Mix by pipetting up and down 8-10 times, and then transfer 100 μ L into wells A2 and B2. Mix and continue the doubling dilution scheme across the plate to wells A10 and B10. Remove and discard 100 μ L from wells A10 and B10 (100 μ L will remain).
The assay buffer in wells A11, B11 and A12, B12 will serve as **Blanks**.
Samples: add 20 μ L of sample to wells C1-H1. Mix by pipetting up and down 8-10 times. Transfer 100 μ L to wells C2-H2. Continue mixing and transferring to column 12. Remove and discard 100 μ L from wells C12-H12 (100 μ L will remain). When finished preparing the plate, the final volume in all wells should be 100 μ L.
5. Cover the plate and incubate for 1 hour \pm 10 minutes at room temperature (20-25°C) away from direct sunlight. **Note:** gentle agitation on a plate shaker during incubations may reduce variability.
6. Gently vortex the polyclonal detection antibody and peroxidase-conjugated goat anti-rabbit antibody. Prepare a 1:1,000 dilution mix by adding 11 μ L detection antibody and 11 μ L goat anti-rabbit antibody together in 11mL assay buffer in a reagent reservoir. **Mix thoroughly.** Wash the plate 3x with 150 μ L wash buffer per well. Add 100 μ L of the detection antibody/goat anti-rabbit antibody mix to each well.
7. Incubate the plate for 1 hour \pm 10 minutes at room temperature (20-25°C) away from direct sunlight.
8. Pour the TMB substrate and stop solution into separate reagent reservoirs so they are ready to use in Step 9. Wash the plate 3x with 150 μ L wash buffer per well.
9. Use a **multi-channel** pipette to add 100 μ L TMB to each well and monitor the reaction as the blue color develops. Once OD450 reaches 0.08-0.09 for Standard 1, use a **multi-channel** pipette to add 50 μ L stop solution to each well (the color will change to yellow).
10. Gently tap the plate to ensure homogeneity and measure the absorbance at 450nm within 30 minutes. The OD for Standard 1 should be between 1.2 and 3.5.