



ELISA-Test Allergenbestimmung

Redstone GmbH
Haferwende 1
D-28357 Bremen

Probenahme: redstone Antisporen-Nebel AsN

1. Untersuchungsmethode:

Zu dem Wirkstoff des redstone Antisporen-Nebel AsN wurde der Allergenstandard Asp f1 (Indoor Biotechnologies: ST-AF1) addiert.


Die Bestimmung des Substanzgehaltes erfolgte mittels eines allergenspezifischen Immunoassay-Verfahrens (ELISA-Test) i.A. durch Indoor Biotechnologies, Inc, USA. (s. Anlage)

2. Untersuchungsergebnis:

In der Probenlösung (Wirkstoff: redstone Antisporen-Nebel AsN) mit der Standardaddition des Aspergillus fumigatus Allergens Asp f1 konnte ein um mehr als 95% reduzierter Allergengehalt gegenüber der Ausgangskonzentration festgestellt werden.

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Kassel, 13.12.2004


Dipl.-Ing. Holger Oetzel

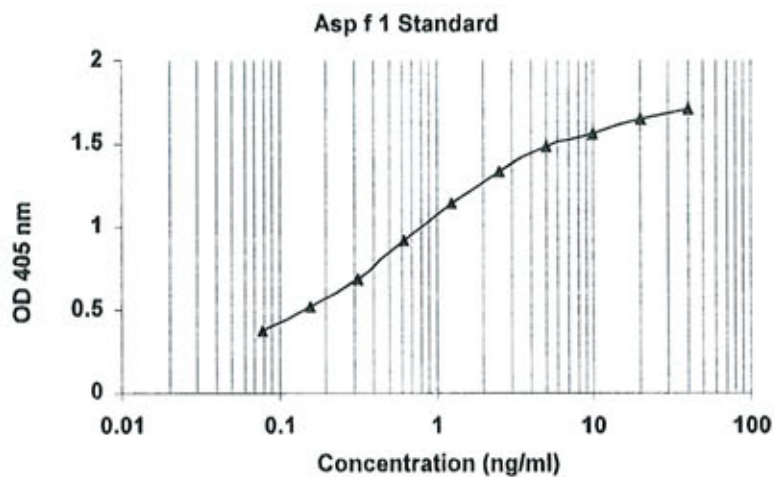
Ende des Prüfberichts

Auszüge des Berichts dürfen nur mit schriftlicher Genehmigung des Prüflaboratoriums veröffentlicht oder vervielfältigt werden. vollständige Kopien sind gestattet. Die Prüfergebnisse beziehen sich ausschließlich auf den untersuchten Prüfgegenstand.



Asp f 1 ELISA kit

Product Code: EL-AF1



Content:

- ◆ Vial 1 (red top) 100 µl
Monoclonal antibody 4A6
Concentration: 10mg/ml in PBS
- ◆ Vial 2 (white top) 400 µl
Asp f 1 Standard
Concentration: 400ng/ml
- ◆ Vial 3 (brown) 100 µl
Polyclonal rabbit anti-Asp f 1 antibody
Dilute: 1:1000 for use

Storage: All reagents should be stored at 4°C

Developed and manufactured by:

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**ELISA protocol for Asp f 1: Product Code EL-AF1**

1. Anti-Asp f 1 mAb 4A6 is supplied HPLC purified as a stock solution in PBS (Product Code: MA-4A6). Dilute the mAb 4A6 1/1000 (i.e. 10µl/10ml) in 50mM carbonate-bicarbonate buffer, pH 9.6. Coat polystyrene microtiter wells (NUNC Maxisorp Cert. NUNC catalog # 439454, Fisher Catalog #12565135) with 100µl of the diluted mAb 4A6 per well. Incubate overnight at 4°C.
 2. Wash wells 3x with PBS-0.05% Tween 20, pH 7.4 (PBS-T). Incubate for 30 min. at room temperature with 100µl 1% BSA PBS-T. Wash 3x with PBS-T.
 3. Add 100µl of diluted allergen standard, house dust or air filter samples. Incubate for 1 hour at room temperature.
 - 3a) Make a Asp f 1 control curve using doubling dilutions of the allergen standard (Product Code: ST-AF1): The control curve dilutions are from 40 - 0.04ng/ml Asp f 1. Pipette 20µl Asp f 1 standard into 180µl 1% BSA PBS-T into wells A1 and B1 of the ELISA plate. Mix well and transfer 100µl across the plate into 100µl 1% BSA PBS-T diluent to make 10 serial doubling dilutions. Wells A11, B11, A12 and B12 should contain only 1% BSA PBS-T as blanks.
 - 3b) House dust samples are routinely diluted two-fold from 1/10-1/80.
 4. Wash wells 3x with PBS-T and add 100µl diluted Rabbit anti-Asp f 1 antibody (Product Code: PA-AF1). The antibody solution contains 50% glycerol and should be diluted 1/1000 (i.e. 10µl/10ml) in 1%BSA-PBS-T. Incubate for 1 hour at room temperature.
 5. Wash wells 3x with PBS-T and add 100µl diluted Peroxidase conjugated Goat anti Rabbit IgG (Jackson Laboratories Cat.# 111-036-046, reconstituted in 1ml distilled water and 1ml glycerol). The reconstituted Goat anti Rabbit IgG should be diluted 1/1000 (i.e. 10µl/10ml).
 6. Wash wells 3x with PBS-T and develop the assays by adding 100µl 1mM ABTS in 70mM citrate phosphate buffer, pH 4.2 containing a 1/1000 dilution of 30% H₂O₂ (i.e. 10µl/10ml ABTS). Read the plate when the optical density at 405nm reaches 2.0-2.4.
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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.
3. Arruda LK, Mann BJ, Chapman MD (1992). Selective expression of a major allergen and cytotoxin, Asp f I, in *Aspergillus fumigatus*. *J. Immunol.*149:3354-3359.
4. Sporik RB, Arruda LK, Woodfolk J, Chapman MD, Platts-Mills TAE. (1993). Environmental exposure to *Aspergillus fumigatus* allergen 1 (Asp f I). *Clin Exp Allergy* 23:326-331.
5. El-Dahr JM, Fink R, Selden R, Arruda LK, Platts-Mills TAE, Heymann PW. (1994). Development of immune responses to *Aspergillus* at an early age in children with cystic fibrosis. *Am J Respir Crit Care Med* 150:1513-1518.



SAMPLE
Certificate of Analysis

Antibody: Monoclonal anti Asp f 1 mAb, 4A6
Immunogen: Asp f 1
Isotype: Mouse IgG1
Specificity: Binds to a common epitope on *Aspergillus fumigatus* allergen, Asp f 1.
Purification: 50% saturated ammonium sulfate fraction of ascites, dialyzed against BBS pH 8.0 for 48 hours.
Concentration: 10mg/ml in phosphate buffered saline, pH 7.2. Based on A280 for IgG (1.42= 1mg/ml) 0.22µm filtered, preservative free.
Lot Number: 2420

Antibody: The rabbit anti-*A.fumigatus* antibody is a polyclonal antiserum raised against partially purified Asp f 1 (a fraction eluted with 2M NaCl from a phenyl-sepharose column) (1, 2).
Specificity: The antiserum contains IgG antibodies to Asp f 1 as well as IgG antibodies to other *Aspergillus* allergens.
Activity: The antiserum has been diluted in phosphate buffered saline, pH 7.4, containing 1% BSA-50%glycerol. The antiserum has been filtered and should be diluted 1/1000 for use in Asp f 1 assays.
Lot Number: 2452

Allergen Standard: Asp f 1
Composition: *Aspergillus* extract prepared in 1% BSA/50% glycerol/PBS. pH 7.4
Concentration: 400ng/ml Asp f 1.
Calibration: Asp f 1 was purified from *Aspergillus* extract by affinity chromatography using anti-Asp f 1 monoclonal antibody 4A6. The protein concentration was determined by ELISA.
Lot Number: 2521