

## Isoform specificity of Indoor Biotechnologies ELISA or MARIA<sup>®</sup> assays

### **Alt a 1 (Product Code EL-AA1):**

No isoforms of Alt a 1 have been identified, though homologs of Alt a 1 are present in other *Alternaria* and mold species.<sup>(1)</sup> The Alt a 1 assay uses a single mAb, 121, which binds to an epitope on each chain of the protein dimer and can be used for allergen capture and detection.

### **Amb a 1 (Product Code EL-AM1):**

This assay uses polyclonal rabbit antibodies to Amb a 1 for allergen capture and detection. The antibodies have been shown to react with all Amb a 1 isoforms by immunoblotting (Dr. Andreas Nandy, Allergopharma, *personal communication*, data on file). Results of the Amb a 1 ELISA have been shown to correlate with FDA radial immunodiffusion values of commercial short ragweed allergenic products.

### **Asp f 1 (Product Code EL-AF1):**

The capture mAb used in this assay, mAb 4A6, also binds to mitogillin, an Asp f 1 homolog from *A. restrictus*. Asp f 1 and Asp r 1 are 95% homologous (differing by 4 amino acid residues) and are antigenically and functionally indistinguishable.<sup>(2)</sup>

### **Bet v 1 (Product Code EL-BV1):**

The assay currently uses a capture mAb 3B4 which binds to Bet v 1A, Bet v 1F, but not Bet v 1D. The detector mAb, 2E10, binds to all isoforms. A replacement capture mAb, 4B10, which binds all isoforms is being tested for use in this product.

### **Der p 1 (Product Code EL-DP1):**

At least 21 isoforms of Der p 1 have been described.<sup>(3)</sup> This assay has not been tested for reactivity with all isoforms. However, crystallographic studies show that the capture mAb (5H8) and detector mAb (4C1) epitopes do not include amino acid substitutions that are involved with isoforms.<sup>(4)</sup>

### **Der f 1 (Product Code EL-DF1):**

Five isoforms of Der f 1 have been described.<sup>(3)</sup> This assay has not been tested for reactivity with all isoforms. However, crystallographic studies show that the detector mAb (4C1) contact residues do not include amino acid substitutions that form isoforms.<sup>(4)</sup>

### **Der p 2 and Der f 2 (Product Code EL-MG2):**

Multiple isoforms of mite Group 2 allergens have been described.<sup>(3)</sup> The capture mAb for the mite Group 2 ELISA, 1D8, binds to Der p 2.0102 and Der p 2.0103, but not to Der p 2.0101. Mab 1D8 binds to Der f 2.0101, Der f 2.0102 and Der f 2.0103. The detector mAb, 7A1, binds to all of the above isoforms. Since the assay was developed, other mite Group 2 isoforms have been described, but these have not been tested in the assay. Mab 1D8 was raised against Der p 2, whereas mAb 7A1 was raised against Der f 2.<sup>(5)</sup> Although the assay is cross-reactive for both Group 2 allergens, the assay has a higher affinity for Der p 2 and separate standards should be used for both assays, when comparing potency of allergic products.<sup>(5)</sup>

**Can f 1 (Product Code EL-CF1):**

The capture mAb for the Can f 1 assay has been shown to bind to Can f 1 from multiple dog breeds, including so-called 'hypoallergenic' breeds.<sup>(6)</sup> Only one sequence, Can f 1.0101, is listed in the WHO/IUIS Allergen nomenclature database ([www.allergen.org](http://www.allergen.org)).<sup>(7)</sup> The detector antibody is a rabbit polyclonal ab.

**Fel d 1 (Product Code EL-FD1):**

Only one sequence, Fel d 1.0101, is listed in the WHO/IUIS Allergen nomenclature database ([www.allergen.org](http://www.allergen.org)). The capture mAb, 6F9, and detector mAb, 3E4, are known to bind Fel d 1 homologs from 'big cat' species. The assay correlates with FDA radial immunodiffusion methods for quantification of Fel d 1.<sup>(8)</sup>

**Phl p 5a (Product Code EL-PP1):**

This assay detects both Phl p 5A and Phl p 5B.

Further references on the antibodies used in Indoor Biotechnologies' immunoassays can be found in the Certificates of Analysis for each assay. Please use the Search function on Indoor Biotechnologies website ([www.inbio.com](http://www.inbio.com)); search using the Product Code and scroll down to the Certificate of Analysis link.

**References**

1. Chruszcz M, Chapman MD, Osinski T, Solberg R, Demas M, Porebski PJ et al. *Alternaria alternata* allergen Alt a 1: A unique beta-barrel protein dimer found exclusively in fungi. *J Allergy Clin Immunol* 2012.
2. Arruda LK, Platts-Mills TA, Fox JW, Chapman MD. *Aspergillus fumigatus* allergen I, a major IgE-binding protein, is a member of the mitogillin family of cytotoxins. *J Exp Med* 1990; 172(5):1529-32.
3. Piboonpocanun S, Malainual N, Jirapongsananuruk O, Vichyanond P, Thomas WR. Genetic polymorphisms of major house dust mite allergens. *Clin Exp Allergy* 2006; 36(4):510-6.
4. Chruszcz M, Pomes A, Glesner J, Vailes LD, Osinski T, Porebski PJ et al. Molecular determinants for antibody binding on group 1 house dust mite allergens. *J Biol Chem* 2012; 287(10):7388-98.
5. Ovsyannikova IG, Vailes LD, Li Y, Heymann PW, Chapman MD. Monoclonal antibodies to group II *Dermatophagoides* spp. allergens: murine immune response, epitope analysis, and development of a two-site ELISA. *J Allergy Clin Immunol* 1994; 94(3 Pt 1):537-46.
6. de Groot H, Goei KG, Van SP, Aalberse RC. Affinity purification of a major and a minor allergen from dog extract: serologic activity of affinity-purified Can f I and of Can f I-depleted extract. *J Allergy Clin Immunol* 1991; 87(6):1056-65.
7. Chapman MD, Pomés A, Breiteneder H, Ferreira F. Nomenclature and structural biology of allergens. *J Allergy Clin Immunol* 2007; 119(2):414-20.
8. Filep S, Tsay A, Vailes LD, Gadermaier G, Ferreira F, Matsui E et al. Specific allergen concentration of WHO and FDA reference preparations measured using a multiple allergen standard. *J Allergy Clin Immunol* 2012; 129(5):1408-10.
9. Filep S, Tsay A, Vailes LD, Gadermaier G, Ferreira F, Matsui EC et al. A multi-allergen standard for immunoassays: CREATE principles applied to eight purified allergens. *Allergy*, In press. 2012.