

## New Developments in Purified Allergens – November 2008

### Mite Der p 1 and Der p 2

Recent improvements in allergen products at Indoor Biotechnologies have focused on increasing the quality of our natural *D. pteronyssinus* allergens (Der p 1 and Der p 2). Specifically, our goals were the removal of endotoxin and serine protease activity, as we have been aware of reports on serine protease contamination in affinity-purified natural Der p 1<sup>(1)</sup>.

- **nDer p 1:** Our modified purification processes include a Benzamidine pre-column to remove trace amounts of serine proteases. The natural Der p 1 produced using this process contains cysteine protease activity and is serine-protease free.
- **nDer p Serine proteases:** The serine proteases from *D. pteronyssinus* are available as a new research reagent. This product may be useful for studies on airway inflammation, asthma, protease activated receptors (PARs), and mouse models of asthma.

### LoTox™

- **LoTox™ nDer p 2:** We offer natural Der p 2 as a new LoTox™ product. LoTox™ allergens are designed for human or murine cellular studies, histamine release assays and animal models of IgE responses.
- **LoTox™ Fel d 1:** Additional new LoTox™ products are natural Fel d 1 as well as recombinant deglycosylated Fel d 1 expressed in *Pichia pastoris*. Using site-directed mutagenesis we have disrupted the N-glycosylation motif of Fel d 1 with a single amino acid substitution (N103Q) to prevent hyperglycosylation seen in recombinant Fel d 1. This genetically engineered and improved rFel d 1 behaves as the structural and antigenic equivalent of natural Fel d 1<sup>(2)</sup>. Data supporting our new products are attached.
- **LoTox™ H22-rFel d 1:** Targeting Fel d 1 to antigen-presenting cells (APCs). The new LoTox™ product H22-rFel d 1, expressed in *Pichia pastoris*, is composed of Fel d 1 linked to the anti-CD64 antibody H22, which binds to the high-affinity IgG receptor, FcγRI (CD64) on APCs<sup>(3)</sup>. This fusion protein induces T-cell subsets characteristic of a protective T-cell response, including T<sub>H</sub>0, regulatory T<sub>H</sub>1 and regulatory T<sub>H</sub>2, in subjects with allergy to cat<sup>(4)</sup>.

### Food Allergens:

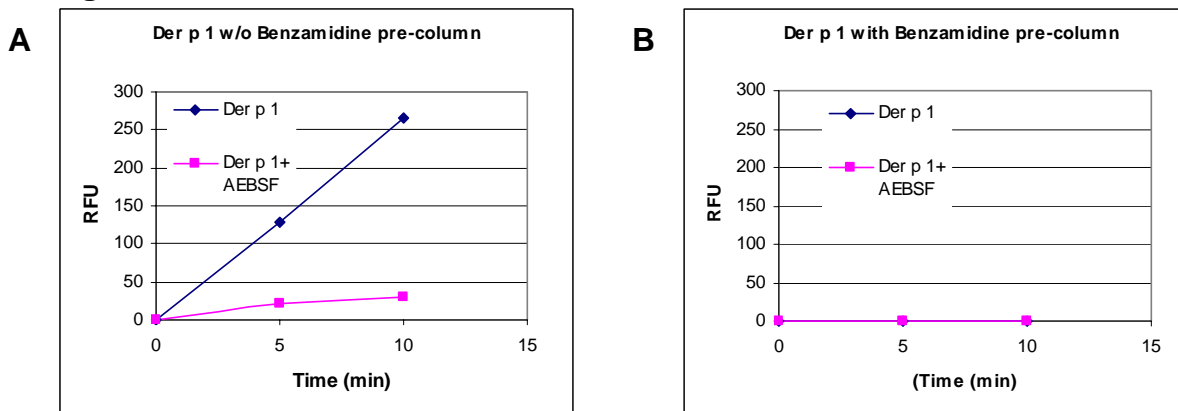
Food allergens are a growing product line at INDOOR Biotechnologies. We are currently offering purified natural peanut allergens Ara h 1 and Ara h 2, as well as natural egg allergens Gal d 1 and Gal d 2. Additional food allergens include Api g 1 (celery), Dau c 1 (carrot) and Mal d 1 (apple).

INDOOR Biotechnologies produces a wide range of highly purified natural and recombinant allergens to meet your research needs. For assistance with allergen products or related research please contact us: Lisa Vailes, MS ([lvailles@inbio.com](mailto:lvailles@inbio.com)) or Sabina Wünschmann, PhD ([sabina@inbio.com](mailto:sabina@inbio.com)), Phone: (434) 984-2304.

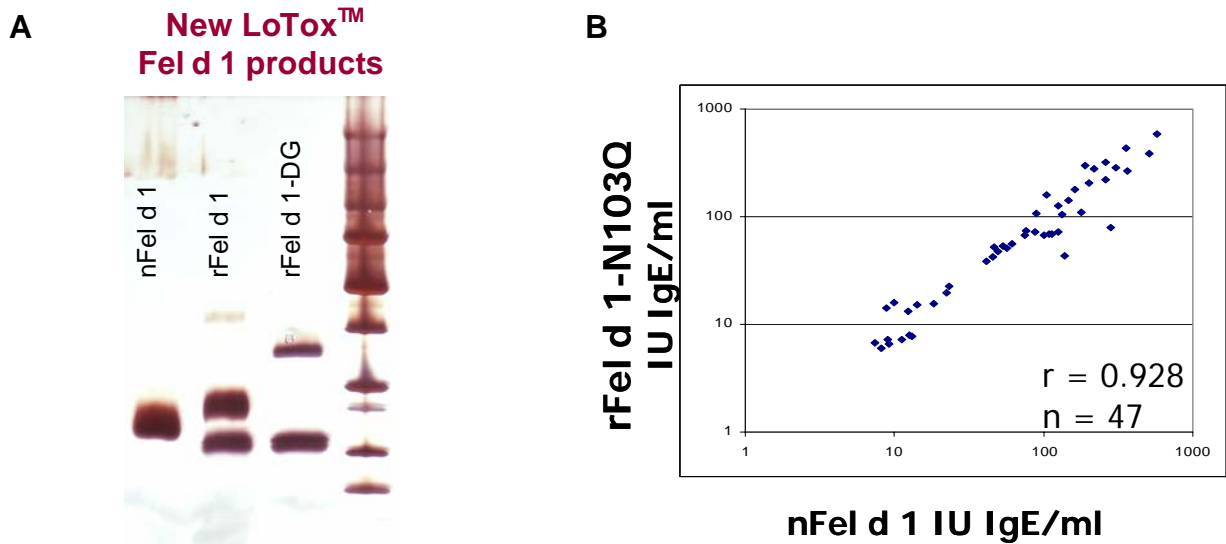
- (1) Takai T, Kato T, Sakata Y, Yasueda H, Izuhara K, Okumura K, Ogawa H. Recombinant Der p 1 and Der f 1 exhibit cysteine protease activity but no serine protease activity. *Biochem Biophys Res Commun.* 2005;328(4):944-52.
- (2) Wünschmann S, Vailes LD, King EM, Aalberse RC, Chapman MD. Expression of a Deglycosylated Recombinant Fel d 1 in *Pichia pastoris*. *J Allergy Clin Immunol* 2008; 121(2):S214.
- (3) Vailes LD, Sun AW, Ichikawa K, Wu Z, Sulahian TH, Chapman MD et al. High-level expression of immunoreactive recombinant cat allergen (Fel d 1): Targeting to antigen-presenting cells. *J Allergy Clin Immunol* 2002; 110(5):757-62.
- (4) Hulse KE, Reefer AJ, Engelhard VH, Satinover SM, Patrie JT, Chapman MD et al. Targeting Fel d 1 to Fc $\gamma$ RI induces a novel variation of the T<sub>H</sub>2 response in subjects with cat allergy. *J Allergy Clin Immunol* 2008;121:756-62.

### Data on New Allergen Products

**Fig. 1**



**Fig.1: Removal of serine protease activity in nDer p 1.** Serine protease activity present in nDer p 1, measured as hydrolysis of the fluorogenic substrate Boc-Gln-Gly-Arg-MCA (A). Benzamidine affinity chromatography removed serine proteases in nDer p 1 (B). AEB SF: serine protease inhibitor.



**Fig. 2 LoTox™ Fel d 1 products. (A)** Silver-stained SDS Page of natural Fel d 1 (nFel d 1), *Pichia pastoris* expressed recombinant (rFel d 1) and deglycosylated recombinant Fel d 1 (rFel d 1-DG). **(B)** Immunoreactivity of Fel d 1, measured using a chimeric ELISA assay for IgE. An excellent correlation was seen between recombinant deglycosylated Fel d 1 (N103Q), expressed in *Pichia* and natural Fel d 1.

**Ordering Information:**

<b>Product Code</b>	<b>Description</b>	<b>Expression System</b>	<b>Unit</b>
LTN-DP2-1	LoTox™ nDer p 2	Natural	250µg
LTN-FD1-1	LoTox™ nFel d 1	Natural	250µg
LTR-FD1D-1	LoTox™ rFel d 1-DG (deglycosylated)	<i>Pichia pastoris</i>	250µg
LTR-FD1H-1	LoTox™ H22-rFel d 1	<i>Pichia pastoris</i>	250µg
NA-DPSP-2	Natural Der p Serine proteases	Natural	500µg