

Recombinant allergens as biomarkers for Timothy grass

Grasses are an important source of inhaled allergens. One of the reasons is the great amount of pollen produced by their species together with their wide distribution in climatic zones which are densely populated.


One of the significant grass allergens is the Phleum pollen (*Phleum pratense*). Up to 20% of allergic individuals worldwide are hypersensitive just to the Phleum and related species of grasses of which numerous cross-reactivities have been reported.

Pollen allergens from various grass species are classified into groups on the basis of their cross-reactivities. **Group I** comprises allergens represented by the major allergen of the Phleum, Phl p 1. The number of Phleum pollinotics hypersensitive to group I varies between 70 to 100% in different countries in Europe. Because of the wide representation of group I allergens in grass, the antibodies against Phl p 1 can be used as a diagnostic marker of the grass allergy.

Another major group of grass allergens is group V with allergens to which 60 to 93% of patients with pollinosis do react. **Group V** is represented by the Phl p 5 molecule.

In addition to major allergens, specific IgE antibodies are directed also against the minor allergens Phl p 7 and Phl p 12.

Recombinant allergens provide new opportunities to refine the diagnostic procedures of IgE mediated allergies. Rekom Biotech, as specialised company in the design and development of recombinant biomarkers, has produced as mature proteins the main allergens of Phleum pollen:

ALLERGEN	REFERENCE	INCIDENCE
Phl p 1	RAL0001 	70%-100%
Phl p 5a	RAL0003 RAL0053BIOT	60%-93%
Phl p 5b	RAL0017	60%-93%
Phl p 7	RAL0002	10%
Phl p 12	RAL0004	20%

These biomarkers have been evaluated in an external study carried out at a Spanish hospital by a group of allergists with positive and negative serum samples from patients. The evaluation of the recombinant allergens has been performed by means of an *in-house* ELISA assay. In this immunoassay, it has been determined the presence of specific IgE in sera that had previously been validated by skin prick testing (SPT) and the UniCAP® test. The sera panel for this study was composed of 25 positive and 10 negative specimen sera.

IgE (IU/ml)	Phl p 1	Phl p 5a	Phl p 5b	Phl p 7	Phl p 12
0,35-0,70	0%	8%	0%	12%	16%
0,70-3,5	8%	8%	8%	28%	16%
3,5-17,50	32%	24%	32%	4%	4%
17,50-50	36%	8%	4%	0%	0%
50-100	0%	4%	8%	0%	0%
>100	16%	8%	4%	0%	0%
Total	92%	60%	56%	44%	36%

This incidence was subsequently compared to the data described by bibliography, finding a very good correlation.

The measure of circulating IgE antibodies specific for a determined allergen provides information about the patient sensitisation to this allergen. In general, low IgE levels would indicate a low probability of developing a clinical disease, while high IgE levels would show a high correlation of developing disease. Through an adequate diagnostic test incorporating our biomarkers, it would be possible to determine the allergen to which the patient is reacting and the levels of specific IgE to this allergen. This quantification will allow to predict more accurately the chance of the patient developing a disease, and thus the need for appropriate treatment.

In the final analysis, these recombinant allergens offer better and more consistent quality than extracts, given that they open the possibility of solving common shortcomings often found when working with extracts, such as cross-reactions and non-reproducibility.