Recombinant allergen Phl p 1 for Phleum pratense (Timothy grass pollen)

CATALOG NUMBER: RAL0001

RECOMBINANT ALLERGEN: Phleum pratense Phl p 1 is the major allergen of grass pollen (Bokanovic et al., 2013). 95% of grass pollen-allergic patients showed IgE binding to Phl p1 assays (Valenta et al., 1992).

DESCRIPTION: the Phleum pratense β-expansin Phl p 1 has been prepared as a recombinant mature allergen fused to a his-tag in its N-terminus.

PRESENTATION: liquid protein solution

SOURCE: Escherichia coli

MOLECULAR WEIGHT: determined by SDS-PAGE, the protein band is between molecular markers of 35,000 and 25,000 Da, while relative molecular mass calculated from amino acid sequence is 32,732.7 Da.

BATCH COMPOSITION:

<table>
<thead>
<tr>
<th>COMPONENTS</th>
<th>COMPOSITION</th>
</tr>
</thead>
<tbody>
<tr>
<td>his-Phl p 1</td>
<td>recombinant allergen with a his-tag</td>
</tr>
<tr>
<td>Storage buffer</td>
<td>20 mM phosphate buffer pH 7 and 0.1 M KCl</td>
</tr>
</tbody>
</table>

QUALITY CONTROL:

1. PROTEIN CONCENTRATION DETERMINED ESPECTROPHOTOMETRICALLY

\[ \text{DO}_{280} = 0.425 \]

**A 0.1% (=1 g/l) = 1.479**

CONCENTRATION*: 0.287 mg/ml

* The measurement of the protein concentration has been performed with the theoretical extinction coefficient of the recombinant protein obtained from Gill and vonHippel, 1989

2. PURITY CONTROL IN SDS-PAGE: 12.5%

![Figure 1. SDS-PAGE analysis (12.5%) of 8 μl of the recombinant allergen Phl p 1. Purity is >95% as determined by gel electrophoresis.](image)

3. ANALYSIS BY AN ELISA ASSAY

This biomarker has 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.

The recombinant allergen Phl p 1 detected 23 positive sera out of 25 (92% incidence) with higher prevalence of sera with titers of 17.50-50 IU/ml (international units per milliliter; 1 IU is equivalent to 2.42 ng of IgE).

4. ABSENCE OF PRECIPITATION AFTER A FREEZING AND THAWING CYCLE: ok

LOT SPECIFICATIONS:

1. CONCENTRATION: 0.287 mg/ml

2. TOTAL QUANTITY PER ALIQUOT: 0.5 mg

3. TOTAL VOLUME PER ALIQUOT: 1.829 ml

4. APPLICATIONS: IgE detection in an ELISA assay. Where this product has not been tested for use in a particular technique, this does not necessarily exclude its use in such procedures. Suggested working dilutions are given as a guide only. It is recommended that the user titrates.

5. STORAGE: protein is shipped with dry ice. Upon arrival, it should be aliquoted in order to avoid repeated freezing and thawing cycles and stored at -20°C to -80°C.

6. OBSERVATIONS: proteins should be maintained frozen at high concentrations. In order to defrost the protein, maintain the aliquot at 25°C without shaking to avoid aggregation. Prior making test dilutions and after defrost the protein, is recommended to remove possible protein aggregates by centrifuging the stock solution, avoiding alterations in the immobilization of the biomolecule to the solid surface.

RELATED PRODUCTS:

Phl p 5a, Phl p 5b, Phl p 7, Phl p 12.

BIBLIOGRAPHY:

Bokanovic et al. Determination of IgE to rPhl p 1 is sufficient to diagnose grass pollen allergy. 2013, Allergy, 68:1403-9.

Valenta R, Vrtala S, Ebner C, Kraft D, Scheiner O. Diagnosis of grass pollen allergy with recombinant timothy


**Important Notes:** During shipment, small volumes of product will occasionally become entrapped in the seal of the product vial. For products with volumes of 200 µl or less, we recommend gently tapping the vial on a hard surface or briefly centrifuging the vial in a tabletop centrifuge to dislodge any liquid in the container's cap.

Although recombinant antigens are expressed in non-pathogenic *E. coli* and bacterial integrity is destroyed during purification, the antigen preparation should be handled as potentially infectious.

**NOT FOR DIAGNOSTIC USE, FOR RESEARCH USE ONLY**