# Recombinant allergen Phl p 7 apo-protein for *Phleum pratense* (Timothy grass pollen)

**CATALOG NUMBER:** RAL0002 **LOT NUMBER:** #

**RECOMBINANT ALLERGEN:** *Phleum pratense* Phl p 7 apoprotein (Niederberger *et al.*, 1999).

**DESCRIPTION:** the *Phleum pratense* polcalcin has been prepared as a recombinant allergen fused to a his-tag. It is produced from the complete ORF of the Phl p 7 gene, which codifies the calcium-binding protein p 7.

**PRESENTATION:** liquid protein solution

**SOURCE:** *Escherichia coli*

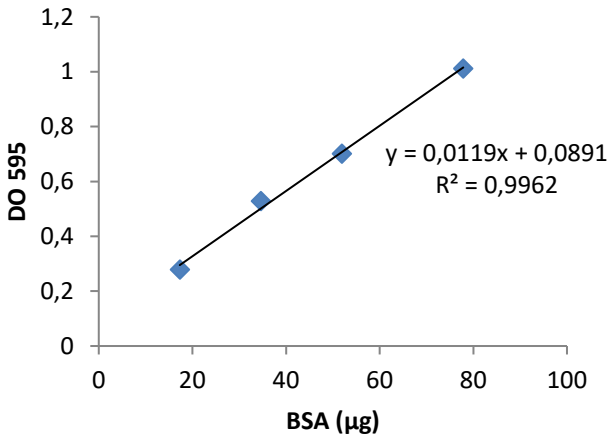
**MOLECULAR WEIGHT:** determined by SDS-PAGE, the protein band is at the molecular marker of 14,400 Da, while relative molecular mass calculated from amino acid sequence is 15,112.6 Da.

**BATCH COMPOSITION:**

| COMPONENTS | COMPOSITION |
|---|---|
| his-apoPhl p 7 | recombinant allergen with a his-tag |
| Storage buffer | 20 mM phosphate buffer pH 8 and 0.15 NaCl and 5 mM EDTA |

**QUALITY CONTROL:**

**1. PROTEIN CONCENTRATION DETERMINED ESPECTROPHOTOMETRICALLY**




This protein does not contain any Trp residues. Experience shows that this could result in more than 10% error in the computed extinction coefficient. Therefore, we have measured the protein concentration by using the colorimetric assay based on the interaction between Coomassie brilliant blue and the arginine and aromatic residues (Bradford Method) and its maximum absorption shifts from 470 nm to 595 nm. The standard curve was performed with the protein BSA. 40 µl of the protein were analyzed.

$DO_{595}$ = 0.977

**2. PURITY CONTROL IN SDS-PAGE: 15%**

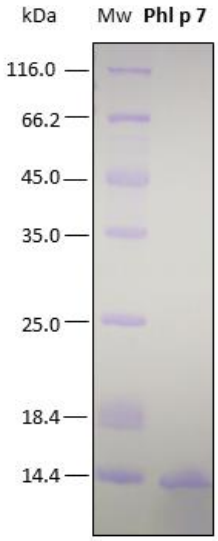



**Figure 1.** SDS-PAGE analysis (15%) of 5 µl of the recombinant allergen. Purity is >95% as determined by gel electrophoresis.

**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 7 detected 11 positive sera out of 25 (44% incidence) with higher prevalence of sera with titers of 0.70-3.5 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:** 1.86 mg/ml

**2. TOTAL QUANTITY PER ALIQUOT:** 1 mg

**3. TOTAL VOLUME PER ALIQUOT**: 0.563 ml

**4. 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 -20ºC to -80ºC.

**5. OBSERVATIONS:** proteins should be maintained frozen at high concentrations. In order to defrost the protein, maintain the aliquot at 25ºC without shaking to avoid aggregation. Prior making test dilutions and after defrosting the protein, is recommended to remove possible protein aggregates by centrifuging the stock solution, avoiding



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alterations in the immobilization of the biomolecule to the solid surface.

**RELATED PRODUCTS:**

Phl p 1, Phl p 5a, Phl p 5a-Biot, Phl p 5b, Phl p 12.

**BIBLIOGRAPHY:**

**Verena Niederberger *et al.*** Calcium-dependent immunoglobulin E recognition of the apo- and calcium-bound form of a cross-reactive two EF-hand timothy grass pollen allergen, Phl p 7. 1999, *FASEB J.*, 13:843-856.

**Bradford, MM.** A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein dye binding. Anal Biochem. 1976, 131:499-503.

**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 containers 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**



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