

Recombinant allergen Phl p 5b for *Phleum pratense* (Timothy grass pollen)

CATALOG NUMBER: RAL0017

LOT NUMBER: #

RECOMBINANT ALLERGEN: *Phleum pratense* Phl p 5 is one of major pollen allergen of timothy grass and accounts for IgE binding in up to 60% of patients (Vrtala *et al.*, 1993). Phl p 5b is one of the isoallergenic groups of Phl p 5 (van Neerven *et al.*, 1992).

DESCRIPTION: the *Phleum pratense* major isoallergen Phl p 5b has been prepared as the recombinant mature allergen fused to a his-tag.

PRESENTATION: liquid protein solution

SOURCE: *Escherichia coli*

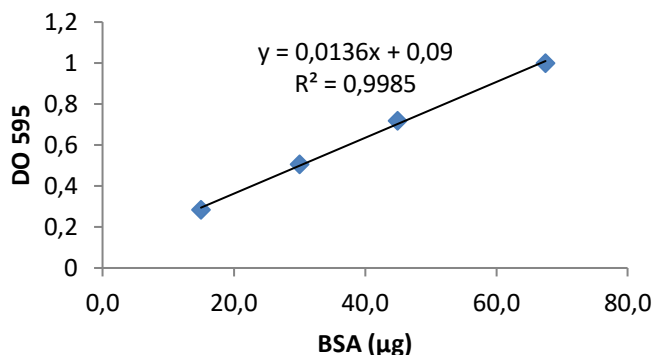
MOLECULAR WEIGHT: determined by SDS-PAGE, the protein band is between molecular markers of 35,000 and 25,000 Da, while relative molecular mass calculated from amino acid sequence is 32,776.99 Da

BATCH COMPOSITION:

COMPONENTS	COMPOSITION
his-Phl p 5b	recombinant allergen with a his-tag
Storage buffer	50 mM MES pH 6.5 and 0.1 M KCl

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.

$DO_{595} = 0.668$
CONCENTRATION = 1-062 mg/ml

2. PURITY CONTROL IN SDS-PAGE: 15%

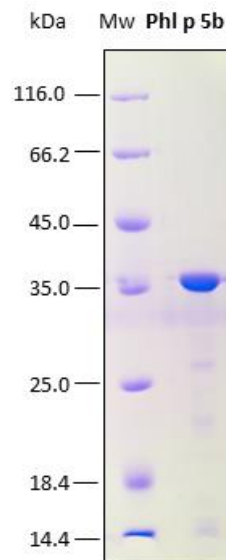


Figure 1. SDS-PAGE analysis (15%) of 1 µl of recombinant allergen. Purity is > 95% as determined by gel electrophoresis.

3. ANALYSIS BY AN ELISA ASSAY

The evaluation of the recombinant allergen has been performed by means of an *in-house* ELISA assay performed in a spanish hospital. This immunoassay was carried out with a serum sample panel of 25 positive and 10 negative specimen sera, pre-validated by skin prick testing (SPT) and the UniCAP® test.

The recombinant allergen Phl p 5b detected 14 positive sera out of 25 (56% incidence) with higher prevalence of sera with titers of 3.5-17.5 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:** 1.062 mg/ml
- 2. TOTAL QUANTITY PER ALIQUOT:** 1 mg
- 3. TOTAL VOLUME PER ALIQUOT:** 0.988 ml
- 4. STORAGE:** Protein is shipped with dry ice. Upon arrival, it should be aliquoted to avoid repeated freezing and thawing cycles and stored at -20°C to -80°C. In order to defrost the protein, maintain the aliquot at 25°C without shaking to avoid aggregation.
- 5. TESTED APPLICATIONS:** ELISA.

6. POSSIBLE APPLICATIONS: WB, DB, Indirect ELISA, positive control in direct ELISA, CLIA, lateral-flow. 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.

7. 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 defrosting 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 1, Phl p 5a, Phl p 5a-Biot, Phl p 7, Phl p 12.

BIBLIOGRAPHY:

Vrtala, S., Sperr, W.R., Reimitzer, I., van Ree, R., Laffer, S., Muller, W.D., Valent, P., Lechner, K., Rumpold, H., Kraft, D. cDNA cloning of a major allergen from timothy grass (*Phleum pratense*) pollen; characterization of the recombinant Phl pV allergen. 1993. J. Immunol. 151, 4773–4781.

van Neerven RJ¹, Wikborg T, Lund G, Jacobsen B, Brinch-Nielsen A, Arved J, Ipsen H. Blocking antibodies induced by specific allergy vaccination prevent the activation of CD4+ T cells by inhibiting serum-IgE-facilitated allergen presentation. 1999. J Immunol. 163:2944-52.

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.

FOR RESEARCH AND COMMERCIAL USE *IN VITRO*: not for human *in vivo* or therapeutic use.