Recombinant allergen Phl p 12 for *Phleum pratense* (Timothy grass pollen)

CATALOG NUMBER: RAL0004 **LOT NUMBER:** #

RECOMBINANT ALLERGEN: *Phleum pratense* Phl p 12 is a profilin allergen responsible for cross-reactivities in about 20% of pollen and food allergic patients (Valenta *et al*., 1994).

DESCRIPTION: the *Phleum pratense* profiling Phl p 12 has been prepared as a recombinant 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 25,000 and 18,400 Da, while relative molecular mass calculated from amino acid sequence is 20,586.2 Da.

BATCH COMPOSITION:

COMPONENTS	COMPOSITION
his-Phl p 12	recombinant allergen with a his-tag
Storage buffer	20 mM phosphate buffer pH 8 and 0.15 M NaCl

QUALITY CONTROL:

1. PROTEIN CONCENTRATION DETERMINED ESPECTROPHOTOMETRICALLY

DO_{280} = 0.935
$A_{0.1\,\%}$ (=1 g/l) = 0.902
CONCENTRATION*: 1.04 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. It is recommended that the users carry out their absorbance determinations to avoid equipment variabilities regarding final concentration, mainly in reproducibility analysis.

2. PURITY CONTROL IN SDS-PAGE: 15%

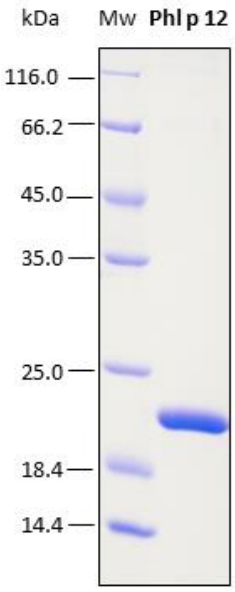

Figure 1. SDS-PAGE analysis (15%) of 3 µl of recombinant allergen. Purity is >98% as determined by gel electrophoresis.

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 12 detected 9 positive sera out of 25 (36% incidence) with higher prevalence of sera with titers of 0.7-3.1 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: 1.04 mg/ml

2. TOTAL QUANTITY PER ALIQUOT: 1 mg

3. TOTAL VOLUME PER ALIQUOT: 1.01 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 -20ºC to -80ºC. In order to defrost the protein, maintain the aliquot at 25ºC without shaking to avoid aggregation.

5. TESTED APPLICATIONS: ELISA.

6. POSIBLE 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 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 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 7.

BIBLIOGRAPHY:

Valenta R, Ball T, Vrtala S, Duchêne M, Kraft D, Scheiner O. cDNA cloning and expression of timothy grass (Phleum pratense) pollen profilin in Escherichia coli:

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comparison with birch pollen profilin. 1994. Biochem Biophys Res Commun. 199:106-18.

Gill SC, von Hippel PH. Calculation of protein extinction coefficients from amino acid sequence data. *Anal Biochem.* 1989 Nov 1;182(2):319-26.

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.

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