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

CATALOG NUMBER: RAL0003BIOT **LOT NUMBER:** #

RECOMBINANT ALLERGEN: *Phleum pratense* Phl p 5a (Hecker *et al*., 2011).

DESCRIPTION: the *Phleum pratense* major allergen Phl p 5 has been prepared as the recombinant mature allergen fused to a his-tag in its N-terminus and monobiotinylated *in vivo.*

PRESENTATION: liquid protein solution

SOURCE: *Escherichia coli*

MOLECULAR WEIGHT: determined by SDS-PAGE, the protein band is at the molecular marker of 45,000 and the dimer is between the molecular markers of 66,200 and 116,000 Da, while relative molecular mass calculated from amino acid sequence is 40,257.84 Da

BATCH COMPOSITION:

COMPONENTS	COMPOSITION
his-Phl p 5	recombinant allergen with a his-tag
Storage buffer	20 mM phosphate buffer pH 6, 0.15 M NaCl and 0.1% polyoxyethylene (10) tridecyl ether

QUALITY CONTROL:

1. PROTEIN CONCENTRATION DETERMINED ESPECTROPHOTOMETRICALLY

DO_{280} = 0.683
$A_{0.1\,\%}$ (=1 g/l) = 0.655
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%

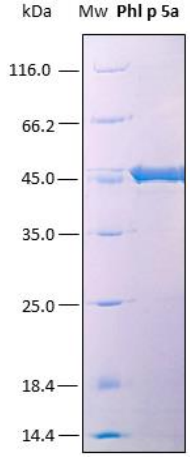

Figure 1. SDS-PAGE analysis (15%) of 3 µl of the recombinant allergen Phl p 5a. Purity is > 95% as determined by gel electrophoresis.

3. WESTERN BLOT WITH STREPTAVIDIN TO DETECT BIOTINYLATION

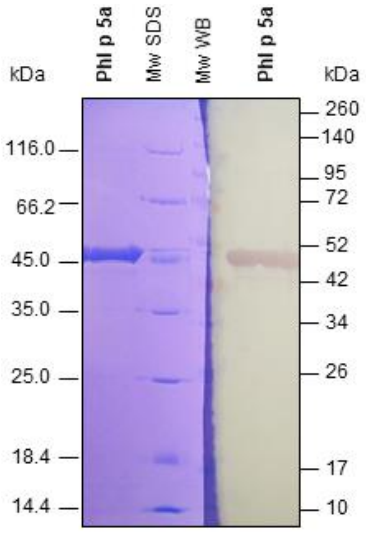

Figure 2. Western blot analysis in order to detect streptavidin /biotin reaction. The incubation was performed with HRP conjugated streptavidin (1:2500)

4. ANALYSIS BY AN ELISA ASSAY

The naked 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 5a detected 15 positive sera out of 25 (60% 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: 1.04 mg/ml

2. TOTAL QUANTITY PER ALIQUOT: 1 mg

3. TOTAL VOLUME PER ALIQUOT: 1 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, plate orientation. Where this product has not been tested for use in a particular technique,

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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, Phl p 12.

BIBLIOGRAPHY:

Julia Hecker *et al*. Generation and epitope analysis of human monoclonal antibody isotypes with specificity for the timothy grass major allergen Phl p 5a. 2011, Molecular Immunology 48:1236-1244.

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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