Recombinant allergen Lep d 2 for *Lepidoglyphus destructor* (storage mite)

CATALOG NUMBER: RAL0008 **LOT NUMBER:** #

RECOMBINANT ALLERGEN: *Lepidoglyphus destructor* Lep d 2 (Varela *et al*., 1994).

DESCRIPTION: Lep d 2 (obsolete name: Lep d 1) is a major allergen of the storage dust mite. The incidence of allergy to *L. destructor* may even surpass that to *Dermatophagoides* in some regions, such as tropical and subtropical areas. This allergen corresponds to the NPC2 family and it has been prepared as a recombinant mature protein fused to a his-tag in its C-terminus.

PRESENTATION: liquid protein solution

SOURCE: *Pichia pastoris*

MOLECULAR WEIGHT: SDS-PAGE analysis determines that the protein band is between the molecular markers of 25,000 Da and 18,400 Da due to the glycosylation pattern, while relative molecular mass, calculated from amino acid sequence and without glycosylation, is 17,026.34 Da.

BATCH COMPOSITION:

COMPONENTS	COMPOSITION
his-Lep d 2	recombinant allergen with a his-tag
Storage buffer	20 mM phosphate buffer pH 6 and 0.25 M KCl

QUALITY CONTROL:

1. PROTEIN CONCENTRATION DETERMINED ESPECTROPHOTOMETRICALLY

DO_{280} = 0.630
$A_{0.1\,\%}$ (=1 g/l) = 0.433
CONCENTRATION*: 1.45 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%

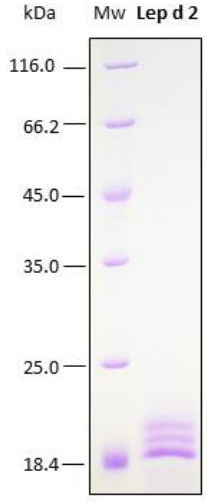

Figure 1. SDS-PAGE analysis (15%) of 5 µl of recombinant allergen. Purity is >95% as determined by gel electrophoresis. The three fragments correspond to the target protein as it has been analyzed in a western blot with an anti-His tag monoclonal antibody.

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 lep d 2 detected 18 positive sera out of 25 (72% 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: 1.45 mg/ml

2. TOTAL QUANTITY PER ALIQUOT: 1 mg

3. TOTAL VOLUME PER ALIQUOT: 0.723 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. **Recombinant allergens expressed in *E. coli* do not contain glycosylation, and *P. pastoris* adds a different glycosylation than the one found in the environment and food allergens. As a result, allergens produced in these expression systems do not need anti-CCD absorbent to eliminate the anti-CCD IgE antibodies present in normal human sera. In the specific case when you measure IgG with an allergen produced in *P. pastoris*, we recommend using our anti-CCD sorbent (Ref. Rekom SOR0001) to avoid cross-reaction with antibodies in sera which react with fungal high mannose glycans.**

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:

Der p 10, Der f 2.

Rekom Biotech S.L. – BIC-Granada, Avda. Innovación, 1 – 18016, Granada (Spain) – Tel: +34 958 63 70 85
E-mail: info@rekombiotech.com – Web: www.rekombiotech.com – An ISO 9001 and 13485 Certified Company

BIBLIOGRAPHY:

Varela, J, Ventas, P, Carreira, J, Barbas, JA, Giménez-gallego G and F Polo. Primary structure of *Lep d* I, the main *Lepidoglyphus destructor* allergen. 1994, Eur. J. Biochem. *225,* 93-98.

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 *P. pastoris* 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.

Rekom Biotech S.L. – BIC-Granada, Avda. Innovación, 1 – 18016, Granada (Spain) – Tel: +34 958 63 70 85
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