Recombinant allergen Art v 3 for *Artemisia vulgaris* (Mugwort pollen)

CATALOG NUMBER: RAL0048 **LOT NUMBER:** #

RECOMBINANT ALLERGEN: *Artemisia vulgaris* Art v 3 is a nonspecific lipid transfer protein type 1 of Mugwort pollen (Gadermaiera *et al*., 2009).

DESCRIPTION: the *Artemisia vulgaris* lipid transfer protein has been prepared as a recombinant mature protein fused to a his-tag.

PRESENTATION: liquid protein solution

SOURCE: *Pichia pastoris*

MOLECULAR WEIGHT: SDS-PAGE analysis determines that the protein band is between the molecular markers of 18,400 Da and 14,400 Da due to the glycosylation pattern, while relative molecular mass, calculated from amino acid sequence and without glycosylation, is 14,045.92 Da.

BATCH COMPOSITION:

COMPONENTS	COMPOSITION
his-Art v 3	recombinant allergen with a his-tag
Storage buffer	20 mM phosphate buffer pH 7, 0.1 M KCl and 5 mM EDTA

QUALITY CONTROL:

1. PROTEIN CONCENTRATION DETERMINED ESPECTROPHOTOMETRICALLY

DO_{280} = 0.96
$A_{0.1\,\%}$ (=1 g/l) = 0.686
CONCENTRATION*: 1.40 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: 17%

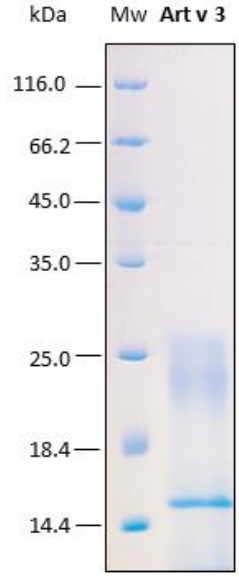
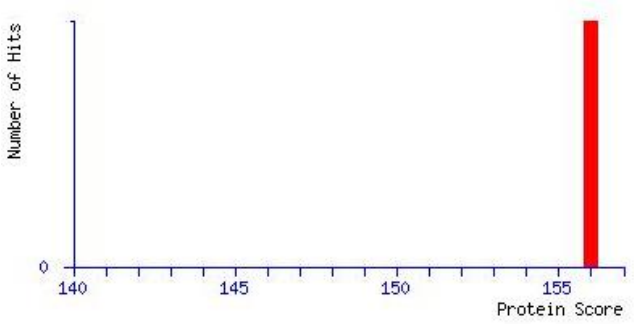


Figure 1. SDS-PAGE analysis (17%) of 5 µl of recombinant allergen. Purity is >95% as determined by gel electrophoresis. The 25 kDa-smear corresponds to the dimer of the protein and the lower band is the monomer. Both bands correspond to the target protein.

3. PROTEIN FINGERPRINT BY MASS SPECTROMETRY

Top Score: 156 for art v 3

Protein score is -10*Log(P), where P is the probability that the observed match is a random event. Protein scores greater than 13 are significant (p<0.05).



Re-Search All Search Unmatched

1. Art **Mass:** 14493 **Score:** 156 **Expect:** 2.5e-016 **Matches:** 11
Art

The MS was performed with a by MALDI TOF/TOF model UltrafleXtreme (Bruker).

4. ABSENCE OF PRECIPITATION AFTER A FREEZING AND THAWING CYCLE: ok

LOT SPECIFICATIONS:

1. CONCENTRATION: 1.40 mg/ml

2. TOTAL QUANTITY PER ALIQUOT: 1 mg

3. TOTAL VOLUME PER ALIQUOT: 0.75 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: none.

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 blocker 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 blocker**

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

(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. The smeary appearance in SDS-PAGE is typical of a different grade of glycosylated protein in gel-development.

RELATED PRODUCTS:

Art v 1, Art v 3 (RAL0006).

BIBLIOGRAPHY:

Gadermaiera, G, Harrer, A, Girbla, T, Palazzoc, P, Himlyb, M, Vogel, O, Brizab, P, Maric, A, and F Ferreira. Isoform identification and characterization of Art v 3, the lipid-transfer protein of mugwort pollen. 2009. Molecular Immunology 46:1919–1924.

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 *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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