

Recombinant biotinylated allergen Tri a 19 for *Triticum aestivum* (wheat)

CATALOG NUMBER: RAL0053BIOT

LOT NUMBER: #

RECOMBINANT ALLERGEN: *Triticum aestivum* (wheat) Tri a 19 (Matsuo *et al.*, 2005).

DESCRIPTION: Tri a 19 or omega-5 gliadin, the major allergen of wheat, has been prepared as a 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 between the molecular markers of 116,000 and 66,200 Da, while relative molecular mass calculated from amino acid sequence is 68,614.82 Da.

BATCH COMPOSITION:

COMPONENTS	COMPOSITION
his-Tri a 19	recombinant allergen with a his-tag
Storage buffer	20 mM phosphate buffer pH 6, 0.15 M NaCl, 0.13 M trehalose, 0.1% polyoxyethylene (10) tridecyl ether and 1 M urea

QUALITY CONTROL:

1. PROTEIN CONCENTRATION DETERMINED ESPECTROPHOTOMETRICALLY

DO₂₈₀ = 0.29
 A_{0.1%} (=1 g/l) = 0.269
 CONCENTRATION*: 1.09 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%

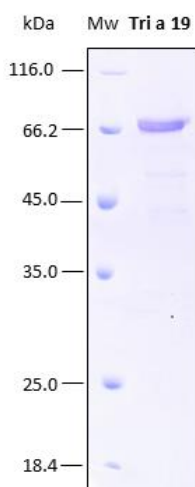


Figure 1. SDS-PAGE analysis (15%) of 6 µl of the recombinant allergen Tri a 19. Purity is > 95% as determined by gel electrophoresis.

3. WESTERN BLOT WITH STREPTAVIDIN TO DETECT BIOTINYLATION

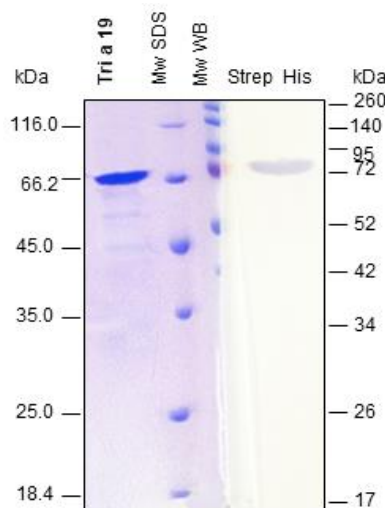


Figure 2. Western blot analysis in order to detect streptavidin /biotin reaction. The incubation was performed with HRP conjugated streptavidin (1:2500)

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

LOT SPECIFICATIONS:

- 1. CONCENTRATION:** 1.09 mg/ml
- 2. TOTAL QUANTITY PER ALIQUOT:** 1 mg
- 3. TOTAL VOLUME PER ALIQUOT:** 0.917 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:** none
- 6. POSSIBLE APPLICATIONS:** WB, DB, Indirect ELISA, positive control in direct ELISA. 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. **If you put the protein on ice and a precipitate appears, it can be urea crystals. In such a case, it would help if you put the protein at room temperature to dissolve the crystals.**

RELATED PRODUCTS:

Tri a 19.

BIBLIOGRAPHY:

Matsuo, H., Kohno, K., and F. Morita. Molecular cloning, recombinant expression and IgE-binding epitope of ω -5 gliadin, a major allergen in wheat-dependent exercise-induced anaphylaxis. 2005, FEBS Journal 272: 4431–4438

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 μ l 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.