



## Recombinant allergen Equ c 1 for *Equus caballus* (Domestic horse)

**CATALOG NUMBER:** RAL0022

**LOT NUMBER:** #

**RECOMBINANT ALLERGEN:** *Equus caballus* Equ c 1 (Gregoire *et al.*, 1996).

**DESCRIPTION:** the *Equus caballus major* allergen, the lipocalin Equ c 1, has been prepared as a recombinant mature allergen fused to a his-tag.

**PRESENTATION:** liquid protein solution

**SOURCE:** *Pichia pastoris*

**MOLECULAR WEIGHT:** determined by SDS-PAGE, the protein band is between molecular markers of 35,000 and 25,000 Da, while relative molecular mass calculated from amino acid sequence and without glycosylation, is 23,488.06 Da.

**BATCH COMPOSITION:**

COMPONENTS	COMPOSITION
his-Equ c 1	recombinant allergen with a his-tag
Storage buffer	20 mM phosphate buffer pH 7, 0.15 M NaCl and 0.1% polyoxyethylene (10) tridecyl ether

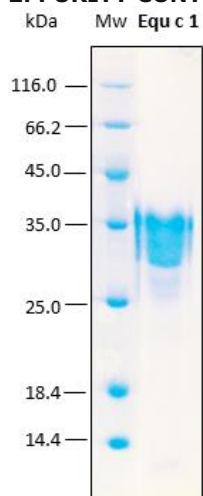
### QUALITY CONTROL:

#### 1. PROTEIN CONCENTRATION DETERMINED ESPECTROPHOTOMETRICALLY

DO<sub>280</sub> = 1.58  
A<sub>0.1%</sub> (=1 g/l) = 0.81  
CONCENTRATION\*: 1.95 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%

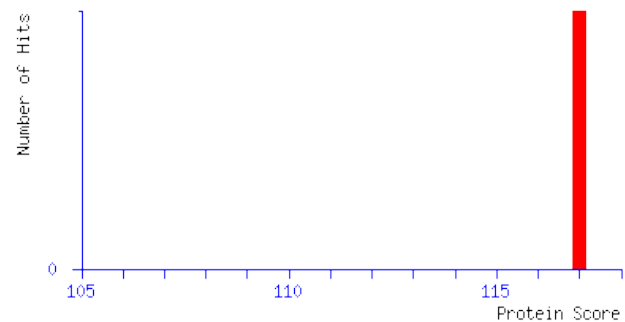


**Figure 1.** SDS-PAGE analysis (15%) of 2  $\mu$ l of recombinant allergen. Purity is >95% as determined by gel electrophoresis.

#### 3. PROTEIN FINGERPRINT BY MASS SPECTROMETRY

Top Score: 146 for equ c 1

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



Ecu\_C\_1 Mass: 23587 Score: 146 Expect: 2.5e-015 Matches: 14

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:** 1.95 mg/ml
- 2. TOTAL QUANTITY PER ALIQUOT:** 1 mg
- 3. TOTAL VOLUME PER ALIQUOT:** 0.538 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, 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 (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 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.

**RELATED PRODUCTS:**

Equ c 1 (RAL0007).

**BIBLIOGRAPHY:**

**Gregoire *et al.*** cDNA cloning and sequencing reveal the major horse allergen Equ c1 to be a glucoprotein member of the lipocalin superfamily. 1996, *J Biol Chem* 1996; 271:32951-9.

**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.**