

Recombinant multi-epitope chimeric antigen CFP10:ESAT6 for *Mycobacterium tuberculosis*

CATALOG NUMBER: RAG0060

LOT NUMBER: #

RECOMBINANT ANTIGEN: *M. tuberculosis* chimeric antigen CFP10:ESAT6 (Bekmurzayeva et al., 2013).

DESCRIPTION: *Mycobacterium tuberculosis* genes for the 10 kDa culture filtrate antigen EsxB (CFP10) and the ESAT6 protein have been expressed as a recombinant multi-epitope chimeric antigen fused to a his-tag in its N-terminus.

PRESENTATION: liquid protein solution

SOURCE: *Escherichia coli*

SPECIFIC ANTIBODY (CALIBRATOR): Polyclonal antibody for Tuberculosis (Rekom Biotech catalog reference PAB0009)

MOLECULAR WEIGHT: determined by SDS-PAGE, the protein band is between molecular markers of 25,000 and 18,400 Da, while relative molecular mass calculated from amino acid sequence is 22,100.0 Da.

BATCH COMPOSITION:

COMPONENTS	COMPOSITION
his-CFP10:ESAT6	recombinant chimeric antigen with a his-tag in its N-terminus
Storage buffer	20 mM phosphate buffer pH 8 and 0.15 M NaCl

QUALITY CONTROL:

1. PROTEIN CONCENTRATION DETERMINED ESPECTROPHOTOMETRICALLY

$DO_{280} = 2.53$

$A_{0.1\%} (=1 \text{ g/l}) = 1.13$

CONCENTRATION*: 2.23 mg/ml

* The measurement of the protein concentration has been performed with the theoretical extinction coefficient of the recombinant protein obtained from Gill and von Hippel, 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%

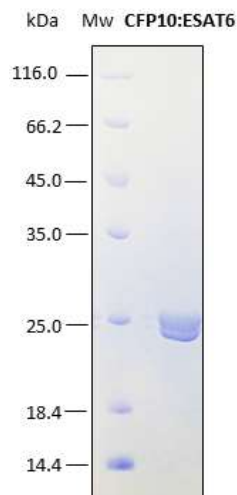


Figure 1. SDS-PAGE analysis (15%) of 2 μ l of recombinant CFP10:ESAT6. Purity is approx. 95% as determined by gel electrophoresis. The band which appears just below, corresponds to an *in vivo* N-terminus or C-terminus degradation product of this same protein, as it is showed in a western blot performed with a his-tag monoclonal antibody.

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

LOT SPECIFICATIONS:

1. CONCENTRATION: 2.23 mg/ml

2. TOTAL QUANTITY PER ALIQUOT: 1 mg

3. TOTAL VOLUME PER ALIQUOT: 0.470 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.

7. OBSERVATIONS: proteins should be maintained frozen at high concentrations. The dilution to be performed for ELISA assays should be made with a small quantity of protein, the same day of the experiment. 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:

CFP10.

BIBLIOGRAPHY:

Bekmurzayeva A., Sypabekova M. and D. Kanayeva.

Tuberculosis diagnosis using immunodominant, secreted antigens of *Mycobacterium tuberculosis*. 2013. Tuberculosis 93: 381-388.

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.