ESAT-6 Recombinant Protein Reference Standard

Catalog No. NR-14868

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For research use only. Not for human use.

Contributor:

BEI Resources or NIH - TB Vaccine Testing and Research Materials Contract

Manufacturer:

Karen Dobos, PhD., Colorado State University, Fort Collins, Colorado, USA and NIH - TB Vaccine Testing and Research Materials Contract

Product Description:

NR-14868 is a recombinant form of the early secretory antigenic target protein, ESAT-6.[1] The protein sequence consists of amino acid residues 1 to 103 including a hexa-histidine tag at the C-terminus. The recombinant protein was expressed in *Escherichia coli* and purified using standard chromatographic techniques followed by endotoxin removal procedures. NR-14868 has a theoretical molecular weight of approximately 11 kDa. The amino acid sequence of NR-14868 is shown below in Table 1.

Material Provided:

Each vial contains approximately 1 mg of NR-14868 in 10 mM ammonium bicarbonate. The concentration, expressed as mg per mL, is shown on the documentation provided by Colorado State University (CSU).

Note: NR-14868 is soluble in 100 mM to 500 mM aqueous buffered salt solutions, such as phosphate buffered saline. A 10 mM ammonium bicarbonate solution can also be used. The recommended concentration is 2 mg/mL or greater. Please refer to the attached CSU product supplement for additional information.

Packaging/Storage:

NR-14868 was packaged aseptically in cryovials. The product is provided frozen on dry ice and should be stored at -80°C or colder immediately upon arrival. Freeze-thaw cycles should be avoided.

Citation:

Acknowledgment for publications should read "The following reagent was obtained through BEI Resources, NIAID, NIH: ESAT-6 Recombinant Protein Reference Standard, NR-14868."

Biosafety Level: 1

Appropriate safety procedures should always be used with this material. Laboratory safety is discussed in the following publication: U.S. Department of Health and Human Services, Public Health Service, Centers for Disease Control and Prevention, and National Institutes of Health. Biosafety in Microbiological and Biomedical Laboratories. 5th ed. Washington, DC: U.S. Government Printing Office, 2009; see www.cdc.gov/biosafety/publications/bmbl5/index.htm.

Disclaimers:

You are authorized to use this product for research use only. It is not intended for human use.

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References:

1. TubercuList: Rv3875
2. Sørensen, A. L., et al. "Purification and Characterization of a Low-Molecular-Mass T-Cell Antigen Secreted by *Mycobacterium tuberculosis*." Infect. Immun. 63 (1995): 1710-1717. PubMed: 7729876.
3. Harboe, M., et al. "Evidence for Occurrence of the ESAT-6 Protein in *Mycobacterium tuberculosis* and Virulent *Mycobacterium bovis* and for Its Absence in *Mycobacterium bovis* BCG." Infect. Immun. 64 (1996): 16-22. PubMed: 8557334.
4. Skjøt, R. L., et al. "Comparative Evaluation of Low-Molecular-Mass Proteins from *Mycobacterium tuberculosis* Members of the ESAT-6 Family as

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Immunodominant T-Cell Antigens." Infect. Immun. 68 (2000): 214-220. PubMed: 10603390.

5. Singh, A., et al. "Dissecting Virulence Pathways of *Mycobacterium tuberculosis* Through Protein-Protein Association." Proc. Natl. Acad. Sci. U. S. A. 103 (2006): 11346-11351. PubMed: 16844784.

Support Provided by NIAID

Table 1 – Protein Sequence

1	MTEQQWNFAG	IEAAASAIQG	NVTSIHSLLD	EGKQSLTKLA	AAWGGSGSEA
61	YQGVQQKWDA	TATELNNALQ	NLARTISEAG	QAMASTEGNV	TGMFALEHHH
101	HHH				

Non ESAT-6 protein residues are underlined.

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Product supplement for Recombinant Esat-6 Protein (BEI Catalog # NR-14868)

An inquiry of recEsat-6 was performed based on a MALDI-ToF analysis of the product indicating a higher molecular mass than the theorized.

Three lots of recEsat-6 were compared:

1. **The exact product shipped to the client reporting the discrepancy**
2. **An archive product lot**
3. **An additional manufactured lot**

All three samples were analyzed by MALDI-ToF, and consistent with the client's analysis, all 3 demonstrated a higher mass than expected (~1 kDa).

All three samples were analyzed by LC-MS/MS (and queried against the SWISSPROT database) to identify the protein and address concerns of sample contamination. Five-hundred and two (502) spectra were queried, 380 identified an ESAT-6 peptide, and the others hit trypsin (the enzyme used for digestion prior to LC-MS/MS analysis), human keratin, and another human protein.

Summary of Sequence (unmodified) and coverage by LC-MS/MS

MTEQQWNFAGIEAAASAIQGNVTSIHSLLDEGKQSLTKLAAAWGGSGSEAYQGVQQKWDA TATELNNALQNLARTISEAGQAMASTEGNVTGMFA

The highlighted amino acids display coverage identified by SEQUEST database analysis for all 3 lots of recombinant ESAT-6 protein.

We did not include the modified C-terminus for query in our LC-MS/MS analysis; which according to our sequence analysis of the construct includes: FALEHHHHHH.

There remains a discrepancy in mass for recESAT-6, in addition to the tagged C-terminus. This protein should not be modified in *E. coli*; however, the protein may include vector construct at the C-terminus. A *denovo* analysis to further identify the mass difference has been initiated.

Our quality control analysis for NR-14868 will continue to include Western blot and LC-MS/MS analysis to confirm the purity and identity of the reagent.