Recombinant Viral Variola CRMB

Catalog Number: 4778-CR/CF

DESCRIPTION

Source	Mouse myeloma cell line, NS0-derived Ala23-Leu349, with a C-terminal 6-His tag Accession # P34015
N-terminal Sequence Analysis	Ala23
Structure / Form	Disulfide-linked homodimer
Predicted Molecular Mass	36.5 kDa (monomer)

SPECIFICATIONS

SDS-PAGE	55 - 60 kDa, reducing conditions
Activity	Measured by its ability to inhibit the TNF-α mediated cytotoxicity in the L-929 mouse fibroblast cells in the presence of the metabolic inhibitor actinomycin D. Gileva, I.P. *et al.* (2006) Biochim. Biophys. Acta. **1764**:1710. The ED_{50} for this effect, in the presence of 0.25 ng/mL of rhTNF-α, is 0.6-2.4 ng/mL.
Endotoxin Level	<0.01 EU per 1 µg of the protein by the LAL method.
Purity	>95%, by SDS-PAGE under reducing conditions and visualized by silver stain.
Formulation	Lyophilized from a 0.2 µm filtered solution in PBS See Certificate of Analysis for details.

PREPARATION AND STORAGE

Reconstitution	Reconstitute at 100 µg/mL in sterile PBS.
Shipping	The product is shipped at ambient temperature. Upon receipt, store it immediately at the temperature recommended below.
Stability & Storage	**Use a manual defrost freezer and avoid repeated freeze-thaw cycles.** ● 12 months from date of receipt, -20 to -70 °C as supplied. ● 1 month, 2 to 8 °C under sterile conditions after reconstitution. ● 3 months, -20 to -70 °C under sterile conditions after reconstitution.

BACKGROUND

Variola virus is the causative agent of human smallpox. Like other poxviruses, it encodes a variety of molecules that shield virus infected cells from immune clearance. The cytokine response modifiers CRMB, C, D, and E, which are differentially expressed among the poxviruses, function as decoy TNF receptors and block the proinflammtatory and antiviral effects of TNF (1, 2). Of the CRM proteins, Variola virus encodes only CRMB, secreted from virus infected cells as a 90 kDa disulfide linked dimer (3). The N-terminal 112 amino acid (aa) region of CRMB mediates binding to human, mouse, and rat TNF as well as human lymphotoxin-α, and neutralizes the cytolytic effects of TNF (3, 4). The C-terminal 155 aa region of CRMB, known as a SECRET domain (smallpox virus-encoded chemokine receptor), binds the chemokines CCL25, CCL28, CXCL12b, CXCL13, and CXCL14, which are involved in the antiviral immune response (4). Functionally, the SECRET domain interferes with the *in vitro* migration of T cells in response to CCL25 (4). A SECRET domain is also present in CRMD but not in CRMC or CRME. Variola virus CRMB shares 84% - 92% aa sequence identity with camelpox virus, cowpox virus, and monkeypox virus CRMB, but only 21% with vaccinia virus CRMB (which lacks a SECRET domain). The TNF binding domain of CRMB shares 30% and 42% aa sequence identity with comparable regions of human TNF R1 and R2, respectively.

References:

1. Johnston, J.B. and G. McFadden, 2003, J. Virol. **77**:6093.
2. Massung, R.F. *et al.* (1993) Nature **366**:748.
3. Gileva, I.P. *et al.* (2006) Biochim. Biophys. Acta **1764**:1710.
4. Alejo, A. *et al.* (2006) Proc. Natl. Acad. Sci. **103**:5995.

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