

DESCRIPTION

Source	<i>Spodoptera frugiperda</i> , Sf21 (baculovirus)-derived human Methionine Aminopeptidase 1/METAP1 protein His52-Phe386 & Lys53-Phe386, with a C-terminal 10-His tag Accession # P53582
N-terminal Sequence Analysis	His52 & Lys53
Predicted Molecular Mass	38 kDa

SPECIFICATIONS

SDS-PAGE	38 kDa, reducing conditions
Activity	Measure by its ability to remove methionine from a fluorogenic peptide substrate H-Met-Gly-Pro-AMC (Catalog # ES017). The resulting GP-AMC is cleaved by Recombinant Human DPPIV/CD26 (Catalog # 9168-SE). The specific activity is >200 pmol/min/μg, as measured under the described conditions. S
Endotoxin Level	<1.0 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	Supplied as a 0.2 μm filtered solution in Tris, NaCl and Glycerol. See Certificate of Analysis for details.

Activity Assay Protocol

Materials	<ul style="list-style-type: none"> • Activation Buffer: 50 mM HEPES, 0.1 mM CoCl₂, 0.1 M NaCl, pH 7.5 • Assay Buffer: 25 mM Tris, pH 8.0 • Recombinant Human Methionine Aminopeptidase 1/METAP1 (rhMETAP1) (Catalog # 3537-ZN) • Recombinant Human DPPIV/CD26 (rhCD26) (Catalog # 9168-SE) • Substrate Met-Gly-Pro-AMC (Catalog # ES017) • F16 Black Maxisorp Plate (Nunc, Catalog # 475515) • Fluorescent Plate Reader (Model: SpectraMax Gemini EM by Molecular Devices) or equivalent
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Assay	<ol style="list-style-type: none"> 1. Dilute rhMETAP1 to 4 μg/mL in Activation Buffer. 2. Dilute Substrate to 200 μM in Activation Buffer. 3. Combine equal volumes of 4 μg/mL rhMETAP1 and 200 μM Substrate. Include a Substrate Blank containing Activation Buffer in place of rhMETAP1. 4. Incubate reactions for 5 minutes at room temperature. 5. Stop reactions by heating at 95-100 °C for 5 minutes. 6. Cool reactions on ice for 3 minutes and centrifuge briefly. 7. Dilute rhCD26 to 2 ng/μL in Assay Buffer. 8. Load into a black well plate 50 μL of each incubated mixture and add 50 μL 2 ng/μL rhCD26 to each. 9. Incubate at room temperature for 10 minutes. 10. Read at excitation and emission wavelengths of 380 nm and 460 nm (top read), respectively, in endpoint mode. 11. Calculate specific activity using the following formula: $\text{Specific Activity (pmol/min/}\mu\text{g)} = \frac{\text{Adjusted Fluorescence* (RFU)} \times \text{Conversion Factor** (pmol/RFU)}}{\text{Incubation time (min)} \times \text{amount of enzyme (}\mu\text{g)}}$ <p>*Adjusted for Substrate Blank **Derived using calibration standard 7-Amino-4-Methyl-Courmarin (AMC) (Sigma, Catalog # A-9891).</p>
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Final Assay Conditions	<p>Per Well:</p> <ul style="list-style-type: none"> • rhMETAP1: 0.10 μg • rhCD26: 0.10 μg • Substrate: 50 μM
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PREPARATION AND STORAGE

Shipping	The product is shipped with dry ice or equivalent. Upon receipt, store it immediately at the temperature recommended below.
Stability & Storage	<p>Use a manual defrost freezer and avoid repeated freeze-thaw cycles.</p> <ul style="list-style-type: none"> • 6 months from date of receipt, -70 °C as supplied. • 3 months, -70 °C under sterile conditions after opening.

BACKGROUND

The human METAP1 gene encodes Methionine Aminopeptidase 1, a member of the M24 family of metalloproteases. METAPs catalyze the removal of the initiator methionine residue from nascent peptides (1) and are essential for cell growth (2). Inhibition of METAPs provides a novel strategy in developing anti-cancer drugs (3). The purified rhMETAP1 consists of amino acid residues 52/53 to 386. A previous report showed that the N-terminal 89 amino acid region was not essential for catalytic activity (4).

References:

1. Lowther, W.T. and B.W. Matthews (2000) *Biochim. Biophys. Acta.* **1477**:157.
2. Li, X. and Y.H. Chang (1995) *Proc. Natl. Acad. Sci. USA* **92**:12357.
3. Liu, S. *et al.* (1998) *Science* **282**:1324.
4. Adlagatta, A. *et al.* (2005) *Biochemistry* **44**:14741.