Recombinant Cynomolgus Monkey Aminopeptidase PILS/ARTS1 His-tag

Catalog Number: 10237-ZN

DESCRIPTION

Source	Chinese Hamster Ovary cell line, CHO-derived cynomolgus monkey Aminopeptidase PILS/ARTS1 protein Ala37-Leu941 Accession # XP_005557484 with a C-terminal 6-His tag
N-terminal Sequence Analysis	Ala37
Predicted Molecular Mass	104 kDa

SPECIFICATIONS

SDS-PAGE	95-107 kDa, under reducing conditions
Activity	Measured by its ability to cleave the fluorogenic peptide substrate, Leu-AMC. The specific activity is >800 pmol/min/µg, as measured under the described conditions.
Endotoxin Level	<0.10 EU per 1 µg of the protein by the LAL method.
Purity	>95%, by SDS-PAGE visualized with Silver Staining and quantitative densitometry by Coomassie® Blue Staining.
Formulation	Supplied as a 0.2 µm filtered solution in Tris, NaCl and Glycerol. See Certificate of Analysis for details.

Activity Assay Protocol

Materials

- Assay Buffer: 25 mM Tris, pH 8.0
- Recombinant Cynomolgus Monkey Aminopeptidase PILS/ARTS1 His-tag (cynoARTS1) (Catalog # 10237-ZN)
- Substrate: Leu-AMC (Bachem, Catalog # I-1240), 10 mM stock in DMSO
- F16 Black Maxisorp Plate (Nunc, Catalog # 475515)
- Fluorescent Plate Reader (Model: SpectraMax Gemini EM by Molecular Devices) or equivalent

Assay

1. Dilute rcynoARTS1 to 1 µg/mL in Assay Buffer.
2. Dilute Substrate to 600 µM in Assay Buffer.
3. Load in plate 50 µL of 1 µg/mL rcynoARTS1, and start the reaction by adding 50 µL of 600 µM Substrate. Include a Substrate Blank containing 50 µL Assay Buffer and 50 µL of 600 µM Substrate.
4. Read at excitation and emission wavelengths of 380 nm and 460 nm (top read), respectively, in kinetic mode of 5 minutes.
5. Calculate specific activity:

$$\text{Specific Activity (pmol/min/µg)} = \frac{\text{Adjusted } V_{max}\text{* (RFU/min) x Conversion Factor** (pmol/RFU)}}{\text{amount of enzyme (µg)}}$$

*Adjusted for Substrate Blank
**Derived using calibration standard 7-amino, 4-Methyl Coumarin AMC (Sigma, Catalog # A9891)

Final Assay Conditions

Per Well:

- rcynoARTS1: 0.05 µg
- Substrate: 300 µM

PREPARATION AND STORAGE

Shipping	The product is shipped with polar packs. Upon receipt, store it immediately at the temperature recommended below.
Stability & Storage	**Use a manual defrost freezer and avoid repeated freeze-thaw cycles.** • 6 months from date of receipt, -20 to -70 °C as supplied. • 3 months, -20 to -70 °C under sterile conditions after opening.

DATA

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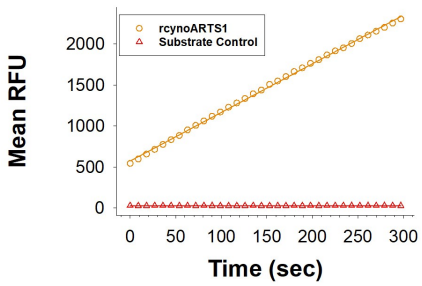
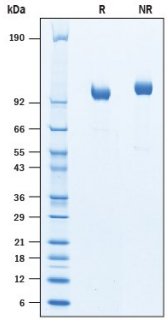
Enzyme Activity



Recombinant Cynomolgus Monkey Aminopeptidase PILS/ARTS1 His-tag (Catalog # 10237-ZN) is measured by its ability to cleave the fluorogenic peptide substrate, Leu-AMC.

SDS-PAGE



2 µg/lane of Recombinant Cynomolgus Monkey Aminopeptidase PILS/ARTS1 His-tag was resolved with SDS-PAGE under reducing (R) and non-reducing (NR) conditions and visualized by Coomassie® Blue staining, showing a band under reducing conditions at 101 kDa.

BACKGROUND

ARTS1 encodes a zinc-dependent metalloprotease (1) known as aminopeptidase PILS (Puromycin-Insensitive Leucyl-Specific), ALAP (adipocyte-derived leucine aminopeptidase), TNFR (type 1 tumor necrosis factor receptor) shedding aminopeptidase regulator, or ERAP1 (endoplasmic reticulum aminopeptidase 1). ARTS1 is widely expressed and known to be present in the endoplasmic reticulum. The protein has four domains with a large cavity between domain II and IV that can accommodate large peptide substrates; the second domain contains the active site and the first domain caps the active site and provides binding sites for the amino terminus of the substrate peptides (2). The enzyme has a unique substrate preference amongst the M1-family of aminopeptidases for longer peptides of up to 16 residues (3, 4) that is consistent with its ability to trim antigen precursors for MHC class 1 presentation (5). Variants and polymorphisms identified in ARTS-1 have linked it to autoimmune disease (6), cancer (7), and hypertension (8). ARTS-1 has been shown to be secreted (9, 10) and cause activation of inflammasome and cathepsin B pathways (11) and consequently to have a direct role in innate immunity and contribute to autoinflammatory and autoimmune diseases (12). ARTS-1 is consequently a potential therapeutic target (12-14).

References:

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