Recombinant Mouse ADAM10

Catalog Number: 946-AD

DESCRIPTION

Source	*Spodoptera frugiperda*, *Sf* 21 (baculovirus)-derived mouse ADAM10 protein Thr215-Glu673, with a C-terminal 10-His tag Accession # O35598
N-terminal Sequence Analysis	Thr215
Structure / Form	Recombinant Mouse ADAM10 is prone to proteolytic cleavage at C-terminus. The predominant form of the purified protein lacks the His tag.
Predicted Molecular Mass	52 kDa

SPECIFICATIONS

SDS-PAGE	60 kDa, reducing conditions
Activity	Measured by its ability to cleave a fluorogenic peptide substrate Mca-PLAQAV-Dpa-RSSSR-NH_2 (Catalog # ES003). The specific activity is >5 pmol/min/µg, as measured under the described conditions.
Endotoxin Level	<1.0 EU per 1 µg of the protein by the LAL method.
Purity	>90%, by SDS-PAGE under reducing conditions and visualized by silver stain.
Formulation	Lyophilized from a 0.2 µm filtered solution in MES, NaCl, $CaCl_2$, $ZnCl_2$, and Brij-35. See Certificate of Analysis for details.

Activity Assay Protocol

Materials

- Assay Buffer: 25 mM Tris, 2.5 µM $ZnCl_2$, 0.005% (w/v) Brij-35, pH 9.0
- Recombinant Mouse ADAM10 (rmADAM10) (Catalog # 946-AD)
- Substrate: MCA-Pro-Leu-Ala-Gln-Ala-Val-DPA-Arg-Ser-Ser-Ser-Arg-NH_2 (Catalog # ES003)
- F16 Black Maxisorp Plate (Nunc, Catalog # 475515)
- Fluorescent Plate Reader (Model: SpectraMax Gemini EM by Molecular Devices) or equivalent

Assay

1. Dilute rmADAM10 to 2 ng/µL in Assay Buffer.
2. Dilute Substrate to 20 µM in Assay Buffer.
3. In a plate load 50 µL of 2 ng/µL rmADAM10, and start the reaction by adding 50 µL of 20 µM Substrate to wells. Include a Substrate Blank containing 50 µL Assay Buffer and 50 µL of 20 µM Substrate.
4. Read at excitation and emission wavelengths of 320 nm and 405 nm (top read), respectively, in kinetic mode for 30 minutes at 37 °C.
5. Calculate specific activity using data from 15-30 minutes:

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

*Adjusted for Substrate Blank

**Derived using calibration standard MCA-Pro-Leu-OH (Bachem, Catalog # M-1975).

Final Assay Conditions

Per Well:

- rmADAM10: 0.1 µg
- Substrate: 10 µM

PREPARATION AND STORAGE

Reconstitution	Reconstitute at 100 µg/mL in sterile 25 mM Tris, pH 7.5.
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.** • 6 months from date of receipt, -20 to -70 °C as supplied. • 3 months, -20 to -70 °C under sterile conditions after reconstitution.

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BACKGROUND

ADAM10 (also known as Kuzbanian, mammalian disintegrin metalloprotease, myelin-associated metalloproteinase) is a member of the ADAM family that contains a disintegrin and metalloprotease-like domain (1, 2). Like other membrane-anchored ADAMs, ADAM10 consists of the following domains, pro with a cysteine switch and furin cleavage sequence, catalytic with the zinc-binding site and Met-turn expected for reprolysins, disintegrin-like, cysteine-rich, EGF-like, transmembrane, and cytoplasmic. ADAM10 is highly conserved, with 97% amino acid identity between mouse, rat, bovine and human and 45% identity between mouse and Drosophila. The active enzyme processes notch, notch ligand delta, and amyloid protein precursor at the alpha site, playing an important role in neurogenesis (3, 4). It also processes the 26 kDa membrane-anchored pro-tumor necrosis factor-α (TNF-α) to the 17 kDa mature TNF-α (5). It cleaves myelin basic protein and type IV collagen (6, 7). ADAM10 is widely expressed in tissues and resides both on the cell surface and in the cell (8, 9).

References:

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