R&D SYSTEMS
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Recombinant Human ADAM10

Catalog Number: 936-AD

DESCRIPTION

Source	*Spodoptera frugiperda*, *Sf* 21 (baculovirus)-derived human ADAM10 protein Thr214-Glu672, with a C-terminal 10-His tag Accession # O14672
N-terminal Sequence Analysis	Thr214
Structure / Form	Recombinant Human ADAM10 may be prone to proteolytic cleavage at C-terminus. The poly-His tag may not be present in the preparation.
Predicted Molecular Mass	52 kDa

SPECIFICATIONS

SDS-PAGE	60 kDa, reducing conditions
Activity	Measured by its ability to cleave a fluorogenic peptide substrate Mca-KPLGL-Dpa-AR-NH_2 (Catalog # ES010). The specific activity is >20 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 visualized with Silver Staining and quantitative densitometry by Coomassie® Blue Staining.
Formulation	Supplied as a 0.2 µm filtered solution in MES, NaCl, $ZnCl_2$,Glycerol and Brij-35. See Certificate of Analysis for details.

Activity Assay Protocol

Materials

- Assay Buffer: 25 mM Tris, 2 µM $ZnCl_2$, 0.005% (w/v) Brij-35, pH 9.0
- Recombinant Human ADAM10 (rhADAM10) (Catalog # 936-AD)
- Fluorogenic Peptide Substrate: MCA-Lys-Pro-Leu-Gly-Leu-DPA-Ala-Arg-NH_2 (Catalog # ES010), 6.2 mM in DMSO
- F16 Black Maxisorp Plate (Nunc, Catalog # 475515)
- Fluorescent Plate Reader (Model: SpectraMax Gemini EM by Molecular Devices) or equivalent

Assay

1. Dilute rhADAM10 to 1 ng/µL in Assay Buffer.
2. Dilute substrate to 20 µM in Assay Buffer.
3. Load into plate 50 µL of 1 ng/µL rhADAM10 and start the reaction by adding 50 µL of 20 µM Substrate. As a control load 50 µL of Assay Buffer and 50 µL of 20 µM Substrate.
4. Seal plate and incubate at 37 °C for 30 minutes.
5. Read at excitation and emission wavelengths of 320 nm and 405 nm (top read), respectively, in endpoint mode.
6. Calculate specific activity:

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

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

Final Assay Conditions

Per Well:

- rhADAM10: 0.050 µg
- Substrate: 10 µM

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	**Use a manual defrost freezer and avoid repeated freeze-thaw cycles.** • 6 months from date of receipt, -70 °C as supplied. • 3 months, -70 °C under sterile conditions after opening.

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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:

1. Rooke, *et al.* (1996) Science **273**:1227.
2. Pan and Rubin (1997) Cell **90**:271.
3. Qi, *et al.* (1999) Science **283**:91.
4. Lammich, *et al.* (1999) Proc. Natl. Acad. Sci. USA **96**:3922.
5. Rosendahl, *et al.* (1997) J. Biol. Chem. **272**:24588.
6. Chantry, *et al.* (1989) J. Biol. Chem. **264**:21603.
7. Millichip, *et al.* (1998) Biochem. Biophys. Res. Comm. **245**:594.
8. Chantry and Glynn (1990) Biochem. J. **268**:245.
9. Fahrenholz, *et al.* (2000) Ann. N.Y. Acad. Sci. **920**:215.

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