Recombinant Human ADAM8

Catalog Number: 1031-AD

DESCRIPTION

Source	Mouse myeloma cell line, NS0-derived human ADAM8 protein Met1-Pro497, with a C-terminal 6-His tag Accession # P78325
N-terminal Sequence Analysis	Glu158
Predicted Molecular Mass	38 kDa

SPECIFICATIONS

SDS-PAGE	42 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 >1 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	Supplied as a 0.2 µm filtered solution in Glycerol, Tris, NaCl and $CaCl_2$. See Certificate of Analysis for details.

Activity Assay Protocol

Materials

- Assay Buffer: 50 mM Tris, 10 mM $CaCl_2$, 150 mM NaCl, pH 7.5 (TCN)
- Recombinant Human ADAM8 (rhADAM8) (Catalog # 1031-AD)
- Bacterial Thermolysin (Catalog # 3097-ZN)
- Phosphoramidon (Catalog # EI006) , 20 mM in methanol
- Fluorogenic Peptide Substrate III: 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 rhADAM8 to 400 µg/mL in Assay Buffer.
2. Combine equal volumes 1.5 µg/mL thermolysin and diluted rhADAM8 so that the final concentration of rhADAM8 is 200 µg/mL and thermolysin is 0.75 µg/mL.
3. Incubate at 37 °C for 30 minutes.
4. Stop reaction by adding Phosphoramidon to a final concentration of 0.05 mM.
5. Incubate at room temperature for 15 minutes.
6. Dilute activated rhADAM8 to 40 ng/µL in Assay Buffer.
7. Dilute substrate to 40 µM in Assay Buffer.
8. Load 50 µL of 40 ng/µL rhADAM8 in a black well plate and start the reaction by adding 50 µL of 40 µM substrate. Include a control containing 50 µL Assay Buffer and 50 µL of 40 µM substrate.
9. Read at excitation and emission wavelengths of 320 nm and 405 nm (top read), respectively in kinetic mode for 5 minutes.
10. 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 MCA-Pro-Leu-OH (Bachem, Catalog # M-1975).

Final Assay Conditions

Per Well:

- rhADAM8: 2.0 µg
- Substrate: 20 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.

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BACKGROUND

ADAM8, also known as cell surface antigen MS2 or CD156a, is a member of the ADAM family that contains a disintegrin and metalloprotease-like domain (1, 2). ADAM8 can cleave a variety of substrates and has been shown as a sheddase for the low affinity IgE receptor CD23 and the neural recognition molecule CHL1 (3, 4). Expression and regulation studies suggest that ADAM8 is a novel osteoclast stimulating factor and may play a role in asthma (5, 6). The 824 amino acid precursor of human ADAM8 consists of a signal peptide (residues 1 to 16), a pro peptide (residues 17 to 199), a metaloprotease domain (residues 200 to 400), a disintegrin-like domain (residues 408 to 494), a cysteine-rich region (residues 497 to 613), an EGF-like domain (residues 614 to 640), a transmembrane region (residues 656 to 676) and a cytoplasmic domain (residues 677 to 824). The purified rhADAM8 (residues 158 to 497) contains a part of the pro peptide and the entire metalloprotease and disintegrin-like domains. It can be activated and assayed under the conditions described in the Activity Assay Protocol.

References:

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