Recombinant Human TACE/ADAM17

Catalog Number: 930-ADB

DESCRIPTION

Source	*Spodoptera frugiperda*, *Sf* 21 (baculovirus)-derived human TACE/ADAM17 protein Arg215-Asn671, with a C-terminal 6-His tag Accession # P78536.1
N-terminal Sequence Analysis	Arg215
Structure / Form	Mature form. Recombinant Human TACE/ADAM17 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	64 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 >500 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 Tris, NaCl 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% Brij-35 (w/v), pH 9.0 (note: It is extremely important that the assay solution does not contain salt ($CaCl_2$, NaCl, Na_2SO_4) because it inhibits TACE activity).
- Recombinant Human TACE/ADAM17 (rhTACE) (Catalog # 930-ADB)
- Substrate: MCA-Pro-Leu-Ala-Gln-Ala-Val-DPA-Arg-Ser-Ser-Ser-Arg-NH_2 (Catalog # ES003) , 2 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 rhTACE to 0.2 ng/µL in Assay Buffer.
2. Dilute Substrate to 20 µM in Assay Buffer.
3. In a plate load 50 µL of 0.2 ng/µL rhTACE and start the reaction by adding 50 µL of 20 µM Substrate. Include a Substrate Blank containing 50 µL Assay Buffer and 50 µL Substrate.
4. Read at excitation and emission wavelengths of 320 nm and 405 nm (top read), respectively in kinetic mode for 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 MCA-Pro-Leu-OH (Bachem, Catalog # M-1975).

Final Assay Conditions

Per Well:

- rhTACE: 0.01 µg
- Substrate: 10 µM

PREPARATION AND STORAGE

Reconstitution	Reconstitute at 0.2 mg/mL in sterile, deionized water.
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 reconstitution.

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BACKGROUND

TACE is a member of the ADAM family that contains A Disintegrin And Metalloprotease-like domain. Like other membrane-anchored ADAMs, TACE consists of a pro domain with a cysteine switch and furin cleavage sequence, a catalytic domain with the zinc-binding site and Met-turn expected for reprolysins, a disintegrin-like domain, a cysteine-rich domain, an EGF-like domain, a transmembrane domain, and the cytoplasmic domain. In addition to its ability to release the 17 kDa extracellular form of tumor necrosis factor-α (TNF-α) from the 26 kDa membrane-anchored TNF-α, TACE also plays an essential role in shedding ectodomains from a variety of proteins such as L-Selectin, Transforming Growth Factor-α, Amyloid Protein Precursor, and Notch-1 receptor. TACE mRNA is present in virtually every tissue and TACE protein resides both on the cell surface and in the cell.

References:

1. Black, R.A. and J.D. Becherer (1998) in *Tumor Necrosis Factor α-Converting Enzyme*. Barrett, A.J. *et al.* (eds): Handbook of Proteolytic Enzymes, San Diego: Academic Press, p. 1315.
2. Primakoff, P. and D.G. Myles (2000) Trends in Genetics **16**:83.

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