Recombinant Human ADAMTS13

Catalog Number: 4245-AD

DESCRIPTION

Source	Chinese Hamster Ovary cell line, CHO-derived human ADAMTS13 protein Gln34 - Trp688 Accession # NP_620594 with a C-terminal 10-His tag
N-terminal Sequence Analysis	Ala75
Predicted Molecular Mass	68 kDa

SPECIFICATIONS

SDS-PAGE	90 kDa, reducing conditions
Activity	Measured by its ability to cleave the fluorogenic peptide substrate, FRETS-VWF73. The specific activity is >10 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 HEPES and NaCl. See Certificate of Analysis for details.

Activity Assay Protocol

Materials

- Assay Buffer: 50 mM Tris, 10 mM $CaCl_2$, 150 mM NaCl, 0.05% (w/v) Brij-35, pH 7.5 (TCNB)
- Recombinant Human ADAMTS13 (rhADAMTS13) (Catalog # 4245-AD)
- Substrate: FRETS-VWF73 (Anaspec, Catalog # 63728), 100 µM 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 rhADAMTS13 to 5 µg/mL in Assay Buffer.
2. Dilute Substrate to 8 µM in Assay Buffer.
3. Load 50 µL of dilute rhADAMTS13 into a plate, and start the reactions by adding 50 µL of 8 µM Substrate. Include a Substrate Blank containing 50 µL of Assay Buffer and 50 µL of 8 µM Substrate.
4. Read at excitation and emission wavelengths of 340 nm and 450 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 FRETS-25-STD1 (Peptides International, Catalog # STD-3720-V).

Final Assay Conditions

Per Well:

- rhADAMTS13: 0.25 µg
- Substrate: 4 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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USA TEL 800 343 7475 Canada TEL 855 668 8722 China TEL +86 (21) 52380373
Europe | Middle East | Africa TEL +44 (0)1235 529449

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BACKGROUND

ADAMTS13 (a disintegrin and metalloproteinase with thrombospondin motifs 13), also known as von Willebrand Factor (vWF) cleaving protease, is a member of the family of secreted zinc proteases with a multi-domain structure (1-3). The protein precursors consist of a signal peptide and following domains: pro, catalytic, disintegrin-like, TS type 1 motif, cysteine-rich, spacer, a second set of seven TSP1 repeats, and two CUB domins. The only known substrate of ADAMTS13 is vWF, a blood glycoprotein with two homeostatic functions (4). It is required for platelet adhesion to sites of vascular damage and acts as a carrier protein for blood-clotting factor VIII in the circulation. It exists in plasma as multimers, the largest of which effectively mediate platelet adhesion. ADAMTS13 cleaves multimeric vWF in the A2 domain at the position, Tyr1605-Met1606. A defect in ADAMTS13 activity is a cause of congenital thrombotic thrombocytopenic purpura (TTP), also known as Upshaw-Schulman syndrome. Lack of ADAMTS13 activity allows unusually large vWF (UlvWF) to occur in plasma (5). These UlvWF multimers have tendency to agglutinate circulating platelets at sites with high levels of shear stress to cause TTP. The purified rhADAMTS13 ends in the spacer domain. The rhvWF-A2 cleaving activity of rhADAMTS13 can be inhibited by 5 mM 1,10-phenanthroline.

References:

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4. Wu, J.J. *et al.* (2006) PNAS. **103**:18470.
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