Recombinant Human ADAMTS13

Catalog Number: 4245-AD

Background

ADAMTS13 (a disintegrin and metalloproteinase with thrombospondin motifs 13), also known as von Willebran 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 starts at the N-terminus of the pro domain and ends in the spacer domain. If desired, the rhvWF-A2 cleaving activity of rhADAMTS13 can be inhibited by 5 mM 1,10-phenanthroline.

References:

1. Furlan, M. *et al.* (1996) Blood. **87**:4223.
2. Porter, S. *et al.* (2005) Biochem. J. **386**:15.
3. Chung, D. W. and J.E. Saddler (2004) in *Handbook of Proteolytic Enzymes*, Barret, A. J. *et al.* eds. pp. 747-751.
4. Wu, J.J. *et al.* (2006) PNAS. **103**:18470.
5. Levy, G.G. *et al.* (2005) Blood. **106**:11.

Description

Source	Chinese Hamster Ovary cell line, CHO-derived Gln34 - Trp688, with a C-terminal 10-His tag Accession # NP_620594
N-terminal Sequence Analysis	No results obtained: Gln34 predicted
Predicted Molecular Mass	73 kDa

Specifications

SDS-PAGE	90 kDa, reducing conditions
Activity	Measured by its ability to cleave recombinant human von Willebrand factor A2 domain (rhvWF-A2; R&D Systems, Catalog # 2764-WF). 0.5 µg of rhADAMTS13 can cleave > 50% of 5 µg of rhvWF-A2 under the described conditions. See Activity Assay Protocol.
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 Sodium Acetate, $CaCl_2$ and NaCl. See Certificate of Analysis for details.

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.

Activity Assay Protocol

Materials

• Assay Buffer: 50 mM Tris, 2 mM $CaCl_2$, 0.01% (w/v) Brij-35, pH 8.5

• Recombinant Human ADAMTS13 (R&D Systems, Catalog # 4245-AD)

• Recombinant Human vWF A2 domain (R&D Systems, Catalog # 2764-WF)

• SDS-PAGE and silver stain reagents or equivalent or Western blot with appropriate antibodies.

Assay

1. Dilute rhvWF-A2 to 200 µg/mL in Assay Buffer.
2. Dilute rhADAMTS13 to 20 µg/mL in Assay Buffer.
3. Mix 25 µL of diluted rhvWF-A2 with 25 µL of diluted rhADAMTS13 in a microtube. Prepare two blanks by mixing 25 µL of diluted rhvWF-A2 with 25 µL of Assay Buffer.
4. Incubate reaction mixtures for 2 hours at 37 °C. Incubate one blank at 37 °C and keep the other blank at –20 °C for 2 hours.
5. Stop the reaction by adding 15 µL of the reaction mixture to 15 µL of SDS-PAGE sample buffer. Treat both blanks with SDS-PAGE sample buffer.
6. Analyze the cleavage products (12 kDa and 7 kDa) by SDS-PAGE (Load 20 µL of the mixture from step 5 per lane (1 µg rhvWF-A2 per lane) followed by silver staining.
7. The cleavage products can also be analyzed by Western blot, loading 0.1 µg per lane of rhADAM13 and using R&D Systems antibodies (MAB27641 or MAB2764).

Final Assay Conditions Per Reaction

• rhADAMTS13: 10 µg/ml

• rhvWF-A2: 100 µg/ml