

DESCRIPTION

Source	Chinese Hamster Ovary cell line, CHO-derived Phe213-Cys685, with a C-terminal 10-His tag Accession # O75173
N-terminal Sequence Analysis	Phe213
Structure / Form	Recombinant Human ADAMTS4 is prone to proteolytic cleavage at C-terminus. The predominant form of the purified protein lacks the His tag.
Predicted Molecular Mass	53 kDa

SPECIFICATIONS

SDS-PAGE	58 kDa, reducing conditions
Activity	Measured by its ability to cleave the fluorogenic peptide substrate, Abz-TEGEARGSVI-Dap(Dnp)-KK-NH ₂ . The specific activity is >7 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 Sodium Acetate, CaCl ₂ and NaCl. See Certificate of Analysis for details.

Activity Assay Protocol

Materials	<ul style="list-style-type: none"> ● Assay Buffer: 50 mM HEPES, 50 mM NaCl, 1 mM CaCl₂, 0.05% Brij-35, pH 7.5 ● Recombinant Human ADAMTS4 (Catalog # 4307-AD) ● Substrate: WAAG-3R (Anaspec, Catalog # 60431-1), 2 mM stock in DMSO ● F16 Black Maxisorp Plate (Nunc, Catalog # 475515) ● Fluorescent Plate Reader (Model: SpectraMax Gemini EM by Molecular Devices) or equivalent
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Assay	<ol style="list-style-type: none"> 1. Dilute rhADAMTS4 to 10 μg/mL in Assay Buffer. 2. Dilute Substrate to 50 μM in Assay Buffer. 3. Load 50 μL of 10 μg/mL of rhADAMTS4 into a plate, and start the reaction by adding 50 μL of 50 μM Substrate. Include a Substrate Blank containing 50 μL of Assay Buffer and 50 μL of Substrate. 4. Read at excitation and emission wavelengths of 340 nm and 420 nm (top read), respectively, in kinetic mode for 5 minutes. 5. Calculate specific activity:
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$$\text{Specific Activity (pmol/min/}\mu\text{g)} = \frac{\text{Adjusted } V_{\text{max}}^* \text{ (RFU/min)} \times \text{Conversion Factor}^{**} \text{ (pmol/RFU)}}{\text{amount of enzyme (}\mu\text{g)}}$$

*Adjusted for Substrate Blank

**Derived using calibration standard Abz-Gly-OH (Bachem, Catalog # E-2920).

Final Assay Conditions	Per Well: <ul style="list-style-type: none"> ● rhADAMTS4: 0.5 μg ● Substrate: 25 μM
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PREPARATION AND STORAGE

Shipping	The product is shipped with polar packs. Upon receipt, store it immediately at the temperature recommended below.
Stability & Storage	<p>Use a manual defrost freezer and avoid repeated freeze-thaw cycles.</p> <ul style="list-style-type: none"> ● 6 months from date of receipt, -20 to -70 °C as supplied. ● 3 months, -20 to -70 °C under sterile conditions after opening.

BACKGROUND

ADAMTS4 (a disintegrin and metalloproteinase with thrombospondin motifs 4), also known as aggrecanase-1, 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 the following domains: pro, catalytic, disintegrin-like, TS type 1 motif, cysteine-rich, and spacer. It is the only ADAMTS identified that has one TS type I motif. It is an active protease effectively cleaving α -2-macroglobulin and aggrecan at multiple sites, and is inhibited by TIMP-3 with inhibition constants in subnanomolar range (4-6). It receives great attention due to the elevation in its mRNA level after treatment with Interleukin-1 (7). However, in a mouse model of osteoarthritis, ADAMTS4 knock-out mice did not exhibit any significant protective effect (8). The purified rhADAMTS4 starts at the catalytic domain and ends before the spacer domain. If desired, the aggrecanase activity can be inhibited by 5 mM 1 10-phenanthroline and Recombinant Human TIMP-3 (Catalog # 973-TM) .

References:

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