

DESCRIPTION

Source Mouse myeloma cell line, NS0-derived human Serpin C1/Antithrombin-III protein
His33-Lys464, with a C-terminal 10-His tag
Accession # P01008

N-terminal Sequence Analysis His33

Predicted Molecular Mass 50 kDa

SPECIFICATIONS

SDS-PAGE 55-65 kDa, reducing conditions

Activity Measured by its ability to inhibit Recombinant Human Coagulation Factor II/Thrombin (Catalog # 1473-SE) cleavage of a fluorogenic peptide substrate Boc-VPR-AMC (Catalog # ES011).
The IC₅₀ value is <5 nM, as measured under the described conditions.

Endotoxin Level <1.0 EU per 1 µg of the protein by the LAL method.

Purity >95%, by SDS-PAGE visualized with Silver Staining and quantitative densitometry by Coomassie® Blue Staining.

Formulation Lyophilized from a 0.2 µm filtered solution in MES and NaCl. See Certificate of Analysis for details.

Activity Assay Protocol

- Materials**
- Assay Buffer: 50 mM Tris, 10 mM CaCl₂, 150 mM NaCl, 0.05% (w/v) Brij-35, pH 7.5 (TCNB)
 - Recombinant Human Serpin C1/Antithrombin-III (rhSerpin C1) (Catalog # 1267-PI)
 - Recombinant Human Coagulation Factor II/Thrombin (Catalog # 1473-SE)
 - Heparin (Sigma, Catalog # H3393), 20 mg/mL stock in deionized water
 - Substrate: BOC-Val-Pro-Arg-AMC (Catalog # ES011), 10 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 Thrombin to 1 µg/mL with Heparin at 48.6 µg/mL in Assay Buffer.
 2. Prepare a curve of rhSerpin C1 (MW: 50,378 Da) in Assay Buffer. Make the following serial dilutions: 1000, 500, 250, 125, 62.5, 41.7, 27.8, 13.9, 6.94, and 2.31 nM.
 3. Mix equal volumes of rhSerpin C1 curve dilutions and Thrombin/Heparin mixture. Include a control (in duplicate) containing equal volumes of Assay Buffer and Thrombin/Heparin mixture.
 4. Incubate reaction mixtures at room temperature for 30 minutes.
 5. After incubation, dilute reaction mixtures by 1/5 in Assay Buffer.
 6. Dilute Substrate to 200 µM in Assay Buffer.
 7. In a plate load 50 µL of the diluted reaction mixtures to wells, and start the reaction by adding 50 µL of 200 µM Substrate.
 8. Read at excitation and emission wavelengths of 380 nm and 460 nm (top read), respectively, in kinetic mode for 5 minutes.
 9. Derive the 50% inhibition concentration (IC₅₀) for rhSerpin C1 by plotting RFU/min (or specific activity) vs. concentration with 4-PL fitting.
 10. Calculate specific activity for Thrombin at each point using the following formula (if needed):

$$\text{Specific Activity (pmol/min/}\mu\text{g)} = \frac{\text{Adjusted } V_{\text{max}}^* \text{ (RFU/min)} \times \text{Conversion Factor}^{**} \text{ (pmol/RFU)}}{\text{amount of enzyme (}\mu\text{g)}}$$

*Adjusted for Substrate Blank

**Derived using calibration standard 7-amino, 4-Methyl Coumarin (Sigma, Catalog # A-9891)

Final Assay Conditions

- Per Well:
- Thrombin: 0.005 µg (1.0 nM)
 - rhSerpin C1: 50, 25, 12.5, 6.25, 3.13, 2.09, 1.39, 0.695, 0.347, and 0.116 nM
 - Substrate: 100 µM

PREPARATION AND STORAGE

Reconstitution Reconstitute at 100 µg/mL in sterile 25 mM MES, 150 mM NaCl, pH 6.5.

Shipping The product is shipped at ambient temperature. 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.

BACKGROUND

Serpin C1 is a member of the Serpin superfamily of the serine protease inhibitors (1). It is the principal plasma Serpin of blood clotting proteases and inhibits thrombin as well as several factors such as Xa (2). Similar to Serpins A5 and D1, its thrombin inhibitory activity is enhanced by heparin. Hereditary and acquired Serpin C1 deficiency is the cause of an increased thrombotic tendency in many cases (3). For example, acquired Serpin C1 deficiency is a common condition in sepsis, after major trauma or surgery (4).

References:

1. Silverman, G.A. *et al.* (2001) *J. Biol. Chem.* **276**:33293.
2. Chuang, Y.-J. *et al.* (2001) *Biochemistry* **40**:6670.
3. Vinazzer, H. (1999) *Semin. Thromb. Hemost.* **25**:257.
4. Risberg, B. (1998) *Blood Coagul. Fibrinolysis Suppl.* **3**:S3.