Recombinant Human Serpin B4

Catalog Number: 9437-PI

DESCRIPTION

Source	*Spodoptera frugiperda*, *Sf* 21 (baculovirus)-derived Asn2-Pro390 Accession # P48594 with an N-terminal Met and 6-His tag
N-terminal Sequence Analysis	Met
Predicted Molecular Mass	46 kDa

SPECIFICATIONS

SDS-PAGE	38-44 kDa, reducing conditions
Activity	Measured by its ability to inhibit chymase cleavage of a fluorogenic peptide substrate, Suc-AAPF-AMC. The IC_{50} is <20 nM, as measured under the described conditions.
Endotoxin Level	<0.10 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	Lyophilized from a 0.2 µm filtered solution in Tris and NaCl. See Certificate of Analysis for details.

Activity Assay Protocol

Materials

- Maturation Buffer: 50 mM MES, pH 5.5
- Cathepsin Buffer: 50 mM MES, 50 mM NaCl, 5 mM DTT, pH 5.5
- Assay Buffer: 20 mM Tris, 2 M KCl, 0.02% (v/v) Triton® X-100, pH 9.0
- Recombinant Human Serpin B4 (rhSerpin B4) (Catalog # 9437-PI)
- Recombinant Human Chymase/CMA1 (rhChymase) (Catalog # 4099-SE)
- Recombinant Mouse Active Cathepsin C/DPPI (rmCathepsin C) (Catalog # 2336-CY)
- Heparin (Tocris, Catalog # 2812), 20 mg/mL stock in deionized water
- Substrate: Suc-Ala-Ala-Pro-Phe-AMC (Bachem, Catalog # I-1465), 10 mM stock in DMSO
- F16 Black Maxisorp Plate (Nunc, Catalog # 475515)
- Fluorescent Plate Reader (Model: Gemini EM by Molecular Devices) or equivalent

Assay

1. Dilute rhChymase-1 to 20 µg/mL in Maturation Buffer.
2. Dilute rmCathepsin C to 20 µg/mL in Cathepsin Buffer.
3. Dilute Heparin to 2.5 mg/mL in deionized water.
4. Combine equal volumes of 20 µg/mL rhChymase and 20 µg/mL rmCathepsin.
5. Add 2.5 mg/mL Heparin to the rhChymase mixture for a final concentration of 49 µg/mL.
6. Incubate rhChymase mixture at room temperature for 1 hour.
7. Dilute the rhChymase mixture to 4 µg/mL of rhChymase in Assay Buffer and incubate at room temperature for 5 minutes.
8. Prepare a curve of rhSerpin B4 (MW: 45,677 Da) in Assay Buffer. Make the following serial dilutions: 2000, 500, 250, 125, 62.5, 31.25, 15.624, 7.812, 3.908 and 0.3908 nM.
9. Combine equal volumes of each point of the rhSerpin B4 curve with 4 µg/mL rhChymase. Include an enzyme control containing equal volumes of Assay Buffer and 4 µg/mL CMA1.
10. Incubate reaction mixtures at room temperature for 30 minutes.
11. Dilute Substrate to 200 µM in Assay Buffer.
12. Load 50 µL of the incubated mixtures into empty wells of a plate, and start the reaction by adding 50 µL of 200 µM Substrate. Include a Substrate Blank containing 50 µL of Assay Buffer and 50 µL of 200 µM Substrate.
13. Read at excitation and emission wavelengths of 380 nm and 460 nm (top read), respectively, in kinetic mode for 5 minutes.
14. Derive the 50% inhibition concentration (IC50) value for rhSerpin B4 by plotting RFU/min (or specific activity) versus concentration with 4-PL fitting.
15. Calculate specific activity for rhChymase at each point using the following formula (if needed):

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

*Adjusted for Substrate Blank.
**Derived using calibration standard 7-amino, 4-Methyl Coumarin (Sigma, Catalog # A9891).

Final Assay Conditions

Per Well:
rhSerpin B4: 500, 125, 62.5, 31.25, 15.625, 7.812, 3.906, 1.953, 0.977, and 0.0977 nM
rhChymase-1: 0.1 µg
Substrate: 100 µM

PREPARATION AND STORAGE

Reconstitution	Reconstitute at 0.5 mg/mL in 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

Serpin B4, also known as squamous cell carcinoma antigen 2 (SCCA-2), is an approximately 45 kDa member of the serpin superfamily of serine protease inhibitors (1). Serpin B4 belongs to the subgroup ovalbumin-related serpins which are involved in the intracellular regulation of apoptosis, inflammation, angiogenesis and embryogenesis (2). Serpin B4 shares 91% sequence identity to Serpin B3, also known as squamous cell carcinoma antigen 1 (SCCA-1), with key differences in the reactive site loops leading to inhibition of distinct classes of proteases (3). Serpin B4 inhibits chymotrypsin-like serine proteases (3). Serpin B4 inhibits apoptosis via STAT activation in an infection (4) and in carcinoma cells (5). It is a mediator in Ras-driven cancer and inflammation (6). Serpin B4 also is a core protein from which Pso p27, an autoantigen present in chronic inflammatory diseases, is derived (7, 8).

References:

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