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
- Asn2-Pro390 Reconstituted Human Serpin B4 (rhSerpin B4) (Catalog # 9437-PI)
- rhChymase (Catalog # 4099-SE)
- 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 Maturation 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 Maturation 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 (IC_{50}) 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):

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

*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.

Rev. 2/6/2018 Page 1 of 2
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: