Recombinant Human Serpin B3/SCCA1

Catalog Number: 6528-PI

DESCRIPTION

Source	*Spodoptera frugiperda*, *Sf* 21 (baculovirus)-derived human Serpin B3/SCCA1 protein Asn2-Pro390, with an N-terminal Met and 6-His tag Accession # P29508
N-terminal Sequence Analysis	Met
Predicted Molecular Mass	45 kDa

SPECIFICATIONS

SDS-PAGE	40-43 kDa, reducing conditions
Activity	Measured by its ability to inhibit active Cathepsin L cleavage of a fluorogenic peptide substrate Z-LR-AMC (Catalog # ES008). The IC_{50} 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 under reducing conditions and visualized by Colloidal Coomassie® Blue stain at 5 µg per lane.
Formulation	Supplied as a 0.2 µm filtered solution in Tris, NaCl and Glycerol. See Certificate of Analysis for details.

Activity Assay Protocol

Materials

- Assay Buffer: 50 mM MES, 5 mM DTT, pH 6.0
- Recombinant Human Serpin B3/SCCA1 (rhSerpin B3) (Catalog # 6528-PI)
- Recombinant Human Cathepsin L (rhCathepsin L) (Catalog # 952-CY)
- Substrate: Z-Leu-Arg-AMC (Catalog # ES008)
- F16 Black Maxisorp Plate (Nunc, Catalog # 475515)
- Fluorescent Plate Reader (Model: Gemini EM by Molecular Devices) or equivalent

Assay

1. Dilute rhCathepsin L to 40 µg/mL in Assay Buffer.
2. Incubate 40 µg/mL rhCathepsin L for 15 minutes on ice to activate.
3. Dilute activated rhCathepsin L to 0.2 µg/mL in Assay Buffer.
4. Prepare a curve of rhSerpin B3 (MW = 45386 Da) in Assay Buffer. Make the following serial dilutions: 1000, 200, 50, 35, 25, 15, 5, 2 and 1 nM.
5. Combine 25 µL of each dilution with 25 µL of the 0.2 µg/mL rhCathepsin L. Include an enzyme control containing Assay Buffer in place of rhSerpin B3.
6. Incubate reaction mixtures at 37 °C for 15 minutes.
7. Dilute reactions by adding 200 µL Assay Buffer to each.
8. Dilute Substrate to 20 µM in Assay Buffer.
9. Load into a black well plate 50 µL of the diluted incubated mixtures and start the reaction by adding 50 µL substrate.
10. Read at excitation and emission wavelengths of 380 nm and 460 nM (top read), respectively, in kinetic mode for 5 minutes.
11. Derive the 50% inhibition concentration (IC_{50}) value for rhSerpin B3 by plotting RFU/min vs. concentration with 4-PL fitting.
12. Calculate the specific activity for rhCathepsin L 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 # A-9891).

Final Assay Conditions

Per Well:

- rhCathepsin L: 0.001 µg
- rhSerpin B3: 50, 10, 2.5, 1.75, 1.25, 0.75, 0.25, 0.1, and 0.05 nM
- Substrate: 10 µM

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.

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BACKGROUND

Serpin B3, also known as squamous cell carcinoma antigen-1 (SCCA-1), is a member of the serpin superfamily of serine protease inhibitors (1). Serpins primarily inhibit serine proteases and some are known to inhibit caspases and papain-like cysteine proteases (1). Serpin B3 belongs to the subgroup ovalbumin-related serpins which are involved in the regulation of apoptosis, inflammation, angiogenesis and embryogenesis (2). Serpin B3 was initially isolated from human cervical squamous cell tissue. It is a tumor marker for squamous cell carcinomas in various areas including the cervix, head, neck and lung (3). Unlike typical serpins, Serpin B3 shows inhibitory activity against papain-like lysosomal cysteine proteases Cathepsin K, L, and S, while lacking inhibitory activity against serine proteases (4).

References:

1. Law, R. *et al.* (2006) Genome Biol. **7**:216.
2. Benarafa, C. and Remold-O'Donnell, E. (2005) Proc. Natl. Acad. Sci. **102**:11367.
3. Higgens, W.J. *et al.* (2010) J. Biol. Chem. **285**:3722.
4. Schick, C. *et al.* (1998) Biochemistry. **37**:5258.

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