Recombinant Mouse Serpin A9/Centerin

Catalog Number: 5679-PI

DESCRIPTION

Source	Mouse myeloma cell line, NS0-derived Asn26-Met418, with an N-terminal 6-His tag Accession # Q9D7D2
N-terminal Sequence Analysis	His
Structure / Form	Monomer
Predicted Molecular Mass	45 kDa

SPECIFICATIONS

SDS-PAGE	40-50 kDa, reducing conditions
Activity	Measured by its ability to inhibit trypsin cleavage of a fluorogenic peptide substrate, Mca-RPKPVE-Nval-WRK(Dnp)-NH_2 (Catalog # ES002). The IC_{50} value is <7 nM, 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 Tris and NaCl. See Certificate of Analysis for details.

Activity Assay Protocol

Materials

- Assay Buffer: 50 mM Tris, 10 mM $CaCl_2$, 0.15 M NaCl, 0.05% (v/v) Brij-35, pH 7.5 (TCNB)
- Recombinant Mouse Serpin A9/Centerin (rmSerpin A9) (Catalog # 5679-PI)
- Recombinant Human Active Trypsin 3/PRSS3 (rhTrypsin 3) (Catalog # 3714-SE)
- Fluorogenic Peptide Substrate: MCA-Arg-Pro-Lys-Pro-Val-Glu-NVAL-Trp-Arg-Lys(DNP)-NH_2 (Catalog # ES002)
- F16 Black Maxisorp Plate (Nunc, Catalog # 475515)
- Fluorescent Plate Reader (Model: SpectraMax Gemini EM by Molecular Devices) or equivalent

Assay

1. Dilute rhTrypsin 3 to 1 ng/µL in Assay Buffer.
2. Prepare a curve of rmSerpin A9 (MW: 44,977 Da) in Assay Buffer. Make the following serial dilutions: 1200 nM, 600 nM, 300 nM, 150 nM, 75 nM, 37.5 nM, 18.8 nM, 9.4 nM, 4.7 nM, and 2.3 nM.
3. Combine equal volumes of dilute rhTrypsin 3 and rmSerpin A9 at each concentration of the curve. Include two controls containing equal volumes of Assay Buffer and diluted rhTrypsin 3 without any rmSerpin A9.
4. Incubate mixtures at 37 °C for 30 minutes.
5. Dilute the reaction mixtures 5-fold with Assay Buffer.
6. Dilute Substrate to 20 µM with Assay Buffer.
7. Load in a plate 50 µL of the incubated mixtures, and start the reaction by adding 50 µL of 20 mM Substrate.
8. Read at excitation and emission wavelengths of 320 nm and 405 nm (top read), respectively, in kinetic mode for 5 minutes.
9. Derive the 50% inhibition concentration (IC_{50}) for rmSerpin A9 by plotting RFU/min (or specific activity) vs concentration with 4-PL fitting.
10. Calculate specific activity of Trypsin at each point using the following formula (if needed):

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

*Adjusted for Substrate Blank

**Derived using calibration standard MCA-Pro-Leu-OH (Bachem, Catalog # M-1975).

Final Assay Conditions

Per Well:

- rhTrypsin 3: 0.005 µg (2.1 nM)
- rmSerpin A9 curve: 60 nM, 30 nM, 15 nM, 7.5 nM, 3.8 nM, 1.9 nM, 0.94 nM, 0.47 nM, 0.23 nM, and 0.12 nM
- Substrate: 10 µM

PREPARATION AND STORAGE

Shipping	The product is shipped with dry ice or equivalent. 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, -70 °C as supplied. ● 3 months, -70 °C under sterile conditions after opening.

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BACKGROUND

Serpin A9, also known as Centerin, is a member of the Serpin superfamily of serine protease inhibitors (1). Serpins are the most broadly distributed superfamily of protease inhibitors. They are known to inhibit serine proteases and some are known to inhibit caspases and papain-like cysteine proteases (1). Recombinant mouse Serpin A9 shows inhibition towards trypsin, thrombin, and plasmin and binds DNA and heparin (2). Its expression is limited to germinal center B cells and lymphoid malignancies with germinal center B-cell maturation (3). New findings suggest that Serpin A9 could be used as a potential diagnostic marker in the recognition of various germinal center derived lymphomas (4).

References:

1. Law, R., *et al.* (2006) Genome Biol. **7**:216.
2. Paterson, M.A., *et al.* (2007) Biochem J. **405:**489.
3. Pan, Z., *et al.* (2003) Am. J. Pathol. **163**:135.
4. Montes-Moreno, S. *et al.* (2008) Blood. **111**:351.

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