Recombinant Mouse Serpin A3N

Catalog Number: 4709-PI

DESCRIPTION

Source	Mouse myeloma cell line, NS0-derived Phe21-Lys418, with a C-terminal 6-His tag Accession # Q91WP6
N-terminal Sequence Analysis	Phe21
Predicted Molecular Mass	46 kDa

SPECIFICATIONS

SDS-PAGE	59 kDa, reducing conditions
Activity	Measured by its ability to inhibit Granzyme B cleavage of *tert*-butoxycarbonyl-Ala-Ala-Asp-thiobenzyl ester (Boc-AAD-SBzl). The IC_{50} is <25 nM, as measured under the described conditions.
Endotoxin Level	<1.0 EU per 1 µg of the protein by the LAL method.
Purity	>85%, 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, pH 7.5
- Activation Buffer: 50 mM MES, 50 mM NaCl, pH 5.5
- Recombinant Mouse Serpin A3N (rmSerpin A3N) (Catalog # 4709-PI)
- Recombinant Human Granzyme B (rhGranzyme B) (Catalog # 2906-SE)
- Recombinant Mouse Active Cathepsin C/DPPI (rmCathepsin C) (Catalog # 2336-CY)
- E-64 (Sigma, Catalog # E-3132), 1 mM stock in DMSO
- DTNB (5,5'-dithio-bis (2-nitrobenzoic acid) (Sigma, Catalog # D-8130), 10 mM stock in DMSO
- Substrate: tert-Butoxycarbonyl-Ala-Ala-Asp-thiobenzyl ester (SM Biochemicals LLC, Catalog # SMSB05), 10 mM stock in DMSO
- 96-well Clear Plate (Costar, Catalog # 92592)
- Plate reader (Model: SpectraMax Plus by Molecular Devices) or equivalent

Assay

1. Activate rhGranzyme B by diluting to 100 µg/mL with 10 µg/mL of rmCathepsin C in Activation Buffer.
2. Incubate at 37 °C for 4 hours.
3. Stop reaction with E-64 at final a concentration of 10 µM in Activation Buffer.
4. Prepare a curve of rmSerpin A3N (MW: 45551 Da) in Assay Buffer. Make the following serial dilutions: 5000, 2500, 1000, 800, 650, 500, 250, 125, 50, and 10 nM.
5. Dilute activated rhGranzyme B to 12.5 µg/mL in Assay Buffer.
6. Combine 20 µL of 12.5 µg/mL rhGranzyme B with 20 µL of the rmSerpin A3N serial curve dilutions. Include two enzyme controls of 20 µL of 12.5 µg/mL rhGranzyme B with 20 µL Assay Buffer.
7. Incubate mixtures at room temperature for 30 minutes.
8. Dilute mixtures by adding 460 µL Assay Buffer to each.
9. Dilute Substrate to 200 µM containing 200 µM of DTNB in Assay Buffer.
10. In a plate load 50 µL of the diluted mixtures into wells.
11. Start the reaction by adding 50 µL of 200 µM Substrate mixture.
12. Read at a wavelength of 405 nm in kinetic mode for 5 minutes.
13. Derive the 50% inhibiting concentration (IC_{50}) for rmSerpin A3N by plotting OD/min (or specific activity) vs. concentration with 4-PL fitting.
14. The specific activity for rhGranzyme B at each point may be determined using the following formula (if needed):

$$\text{Specific Activity (pmol/min/µg)} = \frac{\text{Adjusted } V_{max}^{*} \text{ (OD/min) x well volume (L) x } 10^{12} \text{ pmol/M}}{\text{ext. coeff}^{**} \text{ (M}^{-1}\text{cm}^{-1}\text{) x path corr.}^{***} \text{ (cm) x amount of enzyme (µg)}}$$

*Adjusted for Substrate Blank

**Using the extinction coefficient 13260 $M^{-1}cm^{-1}$

***Using the path correction 0.32 cm

Note: the output of many spectrophotometers is in mOD.

Final Assay Conditions

Per Well:

- rhGranzyme B: 0.025 µg
- rmSerpin A3N curve: 100, 50, 20, 16, 13, 10, 5, 2.5, 1, 0.2, and 0 nM
- Substrate: 100 µM
- DTNB: 100 µM

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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.

BACKGROUND

Serpin A3N is a serine protease inhibitor that is structurally related to α1-antichymotrypsin encoded by the SERPINA3 gene (1). Serpin A3N is highly expressed in brain, testis, lung, thymus, and spleen (2). Serpin A3N secreted by Sertoli cells may regulate the activity of locally produced Granzyme B (3). Granzyme B inhibition by Serpin A3N may therefore regulate Granzyme B-mediated killing by cytotoxic lymphocytes, providing a means to disable cell-mediated immune responses.

References:

1. Forsyth, S. *et al.* (2003) Genomics **81**:336.
2. Horvath, A. J. *et al.* (2004) J. Mol. Evol. **59**:488.
3. Hirst, C. E. *et al.* (2001) Mol. Hum. Reprod. **7**:1133.

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