Recombinant Rat Serpin A12

Catalog Number: 8339-PI

DESCRIPTION

Source	Mouse myeloma cell line, NS0-derived Leu20-Pro411, with a C-terminal 10-His tag Accession # Q8R4Z1
N-terminal Sequence Analysis	Leu20
Predicted Molecular Mass	47 kDa

SPECIFICATIONS

SDS-PAGE	45-54 kDa, reducing conditions
Activity	Measured by its ability to inhibit KLK7 cleavage the fluorogenic peptide substrate, Mca-RPKPVE-Nval-WRK(Dnp)-NH_2 (Catalog # ES002). The IC_{50} is <45 nM, as measured under the described conditions.
Endotoxin Level	<0.10 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 and NaCl. See Certificate of Analysis for details.

Activity Assay Protocol

Materials

- Activation Buffer: 50 mM Tris, 10 mM $CaCl_2$, 150 mM NaCl, 0.05% (w/v) Brij-35, pH 7.5 (TCNB)
- Inhibition Buffer: 25 mM Tris, 150 mM NaCl, pH 7.5
- Assay Buffer: 50 mM Tris, 150 mM NaCl, pH 8.5
- Recombinant Rat Serpin A12 (rrSerpin A12) (Catalog # 8339-PI)
- Recombinant Human Kallikrein 7 (rhKLK7) (Catalog # 2624-SE)
- Bacterial Thermolysin (Catalog # 3097-ZN)
- 1,10 Phenanthroline (Sigma, Catalog # 320056), 0.6 M stock in DMSO
- Substrate: Mca-RPKPVE-Nval-WRK(Dnp)-NH_2 (Catalog # ES002)
- F16 Black Maxisorp Plate (Nunc, Catalog # 475515)
- Fluorescent Plate Reader (Model: Gemini EM by Molecular Devices) or equivalent

Assay

1. Combine rhKLK7 with Thermolysin in Activation Buffer for final concentrations of 100 µg/mL and 10 µg/mL, respectively.
2. Incubate rhKLK7 mixture at 37 °C for 2 hours.
3. Add 1,10 Phenanthroline at a final concentration of 10 mM to stop activation reaction.
4. Prepare a curve of rrSerpin A12 (MW = 46987 Da) in Inhibition Buffer. Make the following serial dilutions: neat, 4000, 2000, 1000, 500, 250, 50, and 5 nM. (Note: High points may not be achievable due to the stock concentration of some lots).
5. Dilute stopped rhKLK7 to 50 µg/mL in Inhibition Buffer.
6. Combine equal volumes of each point of the rrSerpin A12 curve with 50 µg/mL rhKLK7. Include an enzyme control containing equal volumes of Inhibition Buffer and 50 µg/mL rhKLK7.
7. Incubate curve reaction mixtures at room temperature for 30 minutes.
8. Dilute each point of the curve 12.5 fold using Assay Buffer.
9. Dilute Substrate to 20 µM in Assay Buffer.
10. Load 50 µL each of the diluted curve points to a plate, and start the reactions by adding 50 µL of 20 µM Substrate. Include a Substrate Blank containing 50 µL of Assay Buffer and 50 µL of 20 µM Substrate.
11. Read at excitation and emission wavelengths of 320 nm and 405 nm (top read), respectively, in kinetic mode for 5 minutes.
12. Derive the 50% inhibition concentration (IC_{50}) value for rrSerpin A12 by plotting RFU/min (or specific activity) versus concentration with 4-PL fitting.
13. The specific activity for rhKLK7 at each point may be determined using the following formula:

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

*Adjusted for Substrate Blank

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

Final Assay Conditions

Per Well:

- rrSerpin A12: (neat/50), 80, 40, 20, 10, 5, 1, and 0.1 nM
- rhKLK7: 0.1 µg
- 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 A12, also known as Vaspin, is a 45-50 kDa secreted adipokine that contributes to the maintenance of insulin sensitivity (1, 2). It is structurally related to the Serpin family of serine protease inhibitors (3). Mature rat Serpin A12 shares 62% and 88% amino acid sequence identity with human and mouse Serpin A12, respectively (3). It is expressed by adipocytes in visceral and subcutaneous fat, in the gastric glands and epithelium, and in the placenta (3-5). Serpin A12 circulates in a complex with Kallikrein 7, and it prevents the Kallikrein 7 mediated cleavage of Insulin (6). It promotes the elevation of circulating insulin and improves glucose tolerance but can also inhibit the high glucose induced activation of the Insulin Receptor (3, 6, 7). Serpin A12 inhibits TRANCE/RANK L induced osteoclast development and the inflammatory activation of vascular smooth muscle and endothelial cells (7-9). It additionally functions as an anti-apoptotic protein in vascular endothelial cells and osteoblasts (10, 11).

References:

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