Recombinant Mouse Serpin A12

Catalog Number: 8338-PI

DESCRIPTION

Source	*Spodoptera frugiperda*, *Sf* 21 (baculovirus)-derived Leu20-Gly413, with substitutions at Q183R and V403I and a C-terminal 10-His tag Accession # Q7TMF5
N-terminal Sequence Analysis	Leu20
Predicted Molecular Mass	47 kDa

SPECIFICATIONS

SDS-PAGE	43-48 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 <60 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 Mouse Serpin A12 (rmSerpin A12) (Catalog # 8338-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 rmSerpin A12 (MW = 47016 Da) in Inhibition Buffer. Make the following serial dilutions: neat, 8000, 4000, 2000, 1000, 500, 250, 100, and 25 nM. (Note: High points may not be achievable due to the stock concentration of some lots).
5. Dilute activated/stopped rhKLK7 to 50 µg/mL in Inhibition Buffer.
6. Combine equal volumes of each point of the rmSerpin 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 37°C 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 rmSerpin 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:

- rmSerpin A12: (neat/50), 160, 80, 40, 20, 10, 5, 2, and 0.5 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.

bio-techne®
Global bio-techne.com info@bio-techne.com techsupport@bio-techne.com TEL +1 612 379 2956
USA TEL 800 343 7475 Canada TEL 855 668 8722 China TEL +86 (21) 52380373
Europe | Middle East | Africa TEL +44 (0)1235 529449

Recombinant Mouse Serpin A12

Catalog Number: 8338-PI

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 mouse Serpin A12 shares 61% and 88% amino acid sequence identity with human and rat 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:

1. Choi, S.H. et al. (2013) Front. Endocrinol. (Lausanne) **4**:97.
2. Goktas, Z. et al. (2013) Front. Endocrinol. (Lausanne) **4**:69.
3. Hida, K. et al. (2005) Proc. Natl. Acad. Sci. USA **102**:10610.
4. Lee, J.A. et al. (2011) Endocr. J. **58**:639.
5. Caminos, J.E. et al. (2009) Histol. Histopathol. **24**:979.
6. Heiker, J.T. et al. (2013) Cell Mol. Life Sci. **70**:2569.
7. Li, H. et al. (2013} Atherosclerosis **228**:61.
8. Kamino, N. et al. (2013) Connect. Tissue Res. **54**:147.
9. Jung, C.H. et al. (2014) Cardiovasc. Diabetol. **13**:41.
10. Jung, C.H. et al. (2011) Biochem. Biophys. Res. Commun. **413**:264.
11. Zhu, X. et al. (2013) Amino Acids **44**:961.

bio-techne®
Global bio-techne.com info@bio-techne.com techsupport@bio-techne.com TEL +1 612 379 2956
USA TEL 800 343 7475 **Canada** TEL 855 668 8722 **China** TEL +86 (21) 52380373
Europe | Middle East | Africa TEL +44 (0)1235 529449