

## **Recombinant Mouse Serpin A12**

Catalog Number: 8338-PI

Source Source Projectors (SZ 12 (baculovirus)-derived Leu2/C-09/14 with substitutions at 0183R and V403I and a C-terminal 10-His tag Accession # GZTMF5  Nearminal Sequence Leu2/O Analysis  Predicted Molecular 47 kDa  Mass Care Set Molecular 47 kDa  SSE-BIFGATIONS  SSS-RAGE 43-48 kDa, reducing conditions  Activity Measured by its stilling to institi KLK7 cleavage the fluorogenic peptide substrate, Mca-NPKPVE-Nva1-WRK(Drp)-NH <sub>2</sub> (Catalog # ES002). The IC <sub>02</sub> is <60 MJ, as measured under the described conditions.  Endotoxin Level via 10 kD by a 1 μg of the protein by the LAL method.  Purity > 555b, by SSS-PAGE under resource ponditions and visualized by Collodad Coomassie® Blue sitain at 5 μg per lane.  Formulation Supplied as a 0.2 μm filtered solution in Tris and NaCl. Sec Certificate of Analysis for details.  Activity Assay Protocol  Materials  • Activation Buffer: 50 mM Tris, 10 mM NaCl, pt 7.5  • Assay Protocol  **Recombinant Human Kallikrain 1 (rinkLY) (Catalog # 8338-PI)  • Recombinant Human Kallikrain 1 (rinkLY) (Catalog # 2024-SE)  • Backerial Themologins (Catalog # 330058), 0.6 M atock in DIMSO  • Inhibition (Sigma, Catalog # 320058), 0.6 M atock in DIMSO  • Inhibition Hard Recombinant Human Kallikrain 1 (rinkLY) (Catalog # 2024-SE)  • Backerial Themologins (Catalog # 3307-XI)  • 1.10 Phenanthroline (Sigma, Catalog # 320058), 0.6 M atock in DIMSO  • Inhibition Markery Protock or 3.7 for 2 hours.  **Assay Protock  • Floraceant Place Reader (Model) Genmic Mb V) Medicator Devices) or equivalent  **Combine rhiklr's with Themologins (Catalog # 37058)  • Floraceant Place Reader (Model) Genmic Mb V) Medicator Devices) or equivalent  **Protocombinate Mick Place Reader (Model) Genmic Mb V) Medicator Devices or equivalent  **Protocombinate Rhiklr (Model) Genmic Mb V) Medicator Devices or final concentration of 100 µM busines and 100 µM busines an	DESCRIPTION	
Predicted Molecular	Source	Leu20-Gly413, with substitutions at Q183R and V403I and a C-terminal 10-His tag
SPECIFICATIONS  SDS-PAGE 48-RDa, reducing conditions  Activity Measured by its ability to inhibit KLK7 cleavage the fluorogenic peptide substrate, Mca-RPKPVE-Nval-WRK(Dnp)-NH <sub>2</sub> (Catalog # ES002). The IC <sub>20</sub> is 450 Mn.d. as measured under the described conditions.  Endotoxin Level 9-10 Level 1 pg of the protein by the LAL method.  Purity 9-95%, 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 Protect  Materials	•	Leu20
SDS.PAGE   43-48 kDa, reducing conditions		47 kDa
SDS.PAGE   43-48 kDa, reducing conditions	SPECIFICATIONS	
Measured by its ability to inhibit KLK7 cleavage the fluorogenic peptide substrate, Mca-RPKPVE-Nval-WRK(Dnp)-NH- <sub>2</sub> (Catalog # E8002).   The ICo <sub>30</sub> is < 60 nM, as measured under the described conditions.   Endotoxin Level		43-48 kDa, reducing conditions
The ICog is <00 nM, as measured under the described conditions.  Purity		· · · · · · · · · · · · · · · · · · ·
Purity   >55%, by SDS-PAGE under reducing conditions and visualized by Colloidal Coomassie® Blue stain at 5 µg per lane.	Activity	
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 <sub>2</sub> , 150 mM NaCl, 0.05% (w/v) Brij-35, pH 7.5 (TCNB) • Inhibition Buffer: 25 mM Tris, 150 mM NaCl, pH 8.5 • Recombinant Mouse Serpin A12 (mSerpin A12) (Catalog # 838-PI) • Recombinant Human Kallikrein / (RikKT) (Catalog # 824-SE) • Bacterial Thermolysin (Catalog # 3097-ZN) • 110 Phenanthroline (Sigma, Catalog # 320056), 6.6 % stock in DMSO • Substrate: Mea-RPKPVE-Na-INVEK(Dnp)-NH, (Catalog # 26002) • F16 Black Maxiscorp Plate (Nunc, Catalog # 475515) • Fluorescent Plate Reader (Model: Germill 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 or 2 hours. 3. Add 1,10 Phenanthroline at a final concentration of 100 mM to stop activation reaction. 4. Prepare a curve of mSerpin A12 (bm 4 ~ 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 achievated fischeport HkLKT to 51 offul using Assay Buffer. 9. Dilute achievated fischeport HkLKT to 51 offul using Assay Buffer. 10. Load 65 µL each of the curve 12.5 fold using Assay Buffer. 9. Dilute achievated fischeport HkLKT to 51 offul using Assay Buffer. 11. Read at excitation and emission wavelengths of 320 mm and 405 mm (top read), respectively, in kinetic mode for 5 minutes. 12. Derive the 50% inhibition concentration (IC <sub>60</sub> ) value for mSerpin A12 by plotting RFU/min (or specific activity) versus concentration with 4-Pt fitting. 13. The specific activity formUmin/µg) = Adjusted V <sub>max</sub> (RFU/min) x Conversion Factor* (pmol/RFU) amount of enzyme (µ	Endotoxin Level	•
Activity Assay Protocol   Materials		
Activity Assay Protocol  Materials  Activation Buffer: 50 mM Tris, 10 mM CaCl <sub>2</sub> , 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 7.5  Acas pulfer: 50 mM Tris, 150 mM NaCl, pH 7.5  Acas pulfer: 50 mM Tris, 150 mM NaCl, pH 7.5  Acas pulfer: 50 mM Tris, 150 mM NaCl, pH 8.5  Accombinant Mouse Serpin A12 ((mSerpin A12) (Catalog # 8338-PI)  Recombinant Mouse Serpin A12 (mSerpin A12) (Catalog # 2624-SE)  Bacterial Thermolysin (Catalog # 39097-ZN)  1, 10 Phenanthroline (Sigma, Catalog # 390056), 0.6 M stock in DMSO  Substrate: Mea-RPKPVE-Nval-WRK(Drp)NaH, (Catalog # 85002)  F16 Black Maxisory Plate (Nunc, Catalog # 47505)  F16 Black Maxisory Plate (Nunc, Catalog # 47507)  F17 Combine risk (Nunc Marchine)  Acasay  1. Combine risk (Nunc Mitter at 37° C for 2 hours.  3. Add 1,10 Phenanthroline at a final concentration of 10m Mt os top activation reaction.  4. Prepare a curve of mSerpin A12 (WW = 47016 Da) in Inhibition Buffer and 50 to 30 µg/ml. 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).  Dilute activated/Stopped rink Lfx to 50 glyrml. InhikLfx.  5. Dilute activated/Stopped rink Lfx to 50 glyrml. InhikLfx.  7. Incubate curve reaction mixtures at 37° C for 30 minutes.  8. Dilute activated/Stopped rink Lfx to 50 glyrml. InhikLfx.  10. Load 50 µL each for 5 fold using Assay Buffer.  9. Dilute Substrate in 40 glyrml. PickLfx.  11. Read at excitation of the curve 12 5 fold using Assay		
Activation Buffer: 50 mM Tris, 10 mM CaCl <sub>2</sub> , 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 7.5  Recombinant Mouse Serpin A12 (rmSerpin A12) (Catalog # 8338-PI)  Recombinant Mouse Serpin A12 (rmSerpin A12) (Catalog # 8388-PI)  Recombinant Mouse Serpin A12 (rmSerpin A12) (Catalog # 8388-PI)  Bacterial Thermolysin (Catalog # 3097-ZN)  1,10 Phenanthroline (Sigma, Catalog # 3097-ZN)  1,10 Phenanthroline (Sigma, Catalog # 3097-ZN)  Fillo 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, respectively.  Includate rhKLK7 mittura et 37 ° 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 mrserpin A12 curve with 50 μg/mL rhKLK7. Include an enzyme control containing equal volumes of Inhibition Buffer and 50 μg/mL rhKLK7.  7. Includate curve reaction mixtures at 37°C for 30 minutes.  8. Dilute acat point of the curve 12.5 fold using 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.  11. Read at excitation and emission wavelengths of 320 mm and 405 mm (top read), respectively, in kinetic mode for 5 minutes.  22. Derive the 50% inhibition concentration wavelengths of 320 mm and 405 mm (top read), respectively, in kinetic mode for 5 minutes.  23. Adjusted for Substrate Blank  "Derived using calibration standard MCA-Pro-Leu-OH (Bachem, Catalog # M-1975).		
Inhibition Buffer: 25 mM Tris, 150 mM NaCl, pH 7.5  Assay Buffer: 30 mM Tris, 150 mM NaCl, pH 7.5  Recombinant Mouse Serpin A12 (mserpin A12) (Catalog # 8338-PI)  Recombinant Mouse Serpin A12 (mserpin A12) (Catalog # 8338-PI)  Recombinant Mouse Serpin A12 (mserpin A12) (Catalog # 2624-SE)  Bacterial Thermolysin (Catalog # 3097-ZN)  1,10 Phenanthroline (Sigma, Catalog # 30056), 0.6 M stock in DMSO  Substrate: Mcs. PRE/PVE-N-Val-WRK(Drp).NH <sub>2</sub> (Catalog # 85002)  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 mM, (Note: High points may not be achievable due to the tock 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 mserpin 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 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:  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. 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 450 nm (roperator), respec	Activity Assay Protoco	bl
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 ((C <sub>50</sub> ) 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:  Specific Activity (pmol/min/μg) = Adjusted V <sub>max</sub> * (RFU/min) x Conversion Factor** (pmol/RFU) amount of enzyme (μg)  *Adjusted for Substrate Blank **Derived using calibration standard MCA-Pro-Leu-OH (Bachem, Catalog # M-1975).  Final Assay Conditions  PREPARATION AND STORAGE  Shipping  The product is shipped with polar packs. Upon receipt, store it immediately at the temperature recommended below.	Materials	<ul> <li>Inhibition Buffer: 25 mM Tris, 150 mM NaCl, pH 7.5</li> <li>Assay Buffer: 50 mM Tris, 150 mM NaCl, pH 8.5</li> <li>Recombinant Mouse Serpin A12 (rmSerpin A12) (Catalog # 8338-PI)</li> <li>Recombinant Human Kallikrein 7 (rhKLK7) (Catalog # 2624-SE)</li> <li>Bacterial Thermolysin (Catalog # 3097-ZN)</li> <li>1,10 Phenanthroline (Sigma, Catalog # 320056), 0.6 M stock in DMSO</li> <li>Substrate: Mca-RPKPVE-Nval-WRK(Dnp)-NH<sub>2</sub> (Catalog # ES002)</li> <li>F16 Black Maxisorp Plate (Nunc, Catalog # 475515)</li> </ul>
Conditions  • 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.	Assay	<ol> <li>Incubate rhKLK7 mixture at 37 °C for 2 hours.</li> <li>Add 1,10 Phenanthroline at a final concentration of 10 mM to stop activation reaction.</li> <li>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).</li> <li>Dilute activated/stopped rhKLK7 to 50 µg/mL in Inhibition Buffer.</li> <li>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.</li> <li>Incubate curve reaction mixtures at 37°C for 30 minutes.</li> <li>Dilute each point of the curve 12.5 fold using Assay Buffer.</li> <li>Dilute Substrate to 20 µM in Assay Buffer.</li> <li>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.</li> <li>Read at excitation and emission wavelengths of 320 nm and 405 nm (top read), respectively, in kinetic mode for 5 minutes.</li> <li>Derive the 50% inhibition concentration (iC<sub>50</sub>) value for rmSerpin A12 by plotting RFU/min (or specific activity) versus concentration with 4-PL fitting.</li> <li>The specific activity for rhKLK7 at each point may be determined using the following formula:</li> <li>Specific Activity (pmol/min/µg) = Adjusted V<sub>max</sub>* (RFU/min) x Conversion Factor** (pmol/RFU) amount of enzyme (µg)</li> </ol>
Shipping The product is shipped with polar packs. Upon receipt, store it immediately at the temperature recommended below.		<ul> <li>rmSerpin A12: (neat/50), 160, 80, 40, 20, 10, 5, 2, and 0.5 nM</li> <li>rhKLK7: 0.1 μg</li> </ul>
Shipping The product is shipped with polar packs. Upon receipt, store it immediately at the temperature recommended below.	PREPARATION AND ST	TORAGE

Rev. 2/6/2018 Page 1 of 2



6 months from date of receipt, -20 to -70 °C as supplied.
3 months, -20 to -70 °C under sterile conditions after opening.



## **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:

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