Recombinant Human Serpin E2/PN1

Catalog Number: 2980-PI

DESCRIPTION

Source	Mouse myeloma cell line, NS0-derived Met1-Pro397, with a C-terminal 10-His tag Accession # NP_001130000
N-terminal Sequence Analysis	Ser20
Predicted Molecular Mass	43 kDa

SPECIFICATIONS

SDS-PAGE	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 <2 nM, as measured under the described conditions.
Endotoxin Level	<1.0 EU per 1 µg of the protein by the LAL method.
Purity	>95%, by SDS-PAGE under reducing conditions and visualized by silver stain.
Formulation	Supplied as a 0.2 µm filtered solution in Sodium Acetate, NaCl and Glycerol. See Certificate of Analysis for details.

Activity Assay Protocol

Materials

- Assay Buffer: 50 mM Tris, 10 mM $CaCl_2$, 150 mM NaCl, 0.05% (w/v) Brij-35, pH 7.5 (TCNB)
- Recombinant Human Serpin E2/PN1(rhSerpin E2) (Catalog # 2980-PI)
- Trypsin (Sigma, Catalog # T-1426)
- 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 Trypsin to 0.25 µg/mL in Assay Buffer and KEEP ON ICE.
2. Prepare a curve of rhSerpin E2 (MW: 43,200 Da) in Assay Buffer. Make the following serial dilutions: 250, 100, 50, 30, 20, 10, 5, 2.5, and 0.5 nM.
3. Combine equal volumes of diluted Trypsin and rhSerpin E2 at each concentration of the curve. Include two controls containing equal volumes of Assay Buffer and diluted Trypsin without any rhSerpin E2.
4. Incubate at room temperature for 30 minutes.
5. Dilute reaction mixtures five fold in Assay Buffer.
6. Dilute Substrate to 20 µM in Assay Buffer.
7. Load 50 µL of the rhSerpin E2 curve in a plate, and start the reaction by adding 50 µL of 20 µM Substrate to wells.
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 rhSerpin E2 by plotting RFU/min (or specific activity) vs. concentration with 4-PL fitting.
10. The specific activity for Trypsin at each point may be determined using the following formula (if needed):

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

*Adjusted for Substrate Blank

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

Final Assay Conditions

Per Well:

- Trypsin: 0.00125 µg
- rhSerpin E2 curve: 12.5, 5, 2.5, 1.5, 1, 0.5, 0.25, 0.125, and 0.025 nM
- 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 E2, also known as protease nexin I or glial-derived nexin (GDN), is a member of the Serpin superfamily of the serine protease inhibitors (1). Serpin E2 is a potent inhibitor of thrombin, plasmin and plasminogen activators (2). It is differentially expressed during neuronal differentiation and is able to transform human embryonic kidney cells into neuron-like cells (3). Its over-expression in mice leads to progressive neuronal and motor dysfunction in these animals (4). It is also over-expressed in the majority of pancreatic carcinoma as well as gastric and colorectal cancer samples whereas it is weakly expressed in all normal pancreas and chronic pancreatitis tissue samples (5). It plays an important role in controlling male fertility because its knockout male mice show a marked impairment in fertility from the onset of sexual maturity and its abnormal expression is found in the semen of men with seminal dysfunction (6). The deduced amino acid sequence of rhSerpin E2 is the same as that in NP_001130000, which predicts Arg329 in its 397 amino acid residues (7). An alternatively splice form predicts Thr-Gly at positions 329 and 330 in its 398 amino acid sequence (8-10).

References:

1. Silverman, G.A. *et al.* (2001) J. Biol. Chem. **276**:33293.
2. Rossignol, P. *et al.* (2004) J. Biol. Chem. **279**:10346.
3. Lin, H.J. *et al.* (2005) Int. J. Dev. Neurosci. **23**:9.
4. Meins, M. *et al.* (2001) J. Neurosci. **21**:8830.
5. Buchholz, M. *et al.* (2003) Cancer Res. **63**:4945.
6. Murer, V. *et al.* (2001) Proc. Natl. Acad. Sci. USA **98**:3029.
7. Strausberg, R.L. *et al.* (2002) Proc. Natl. Acad. Sci. USA **99**:16899.
8. Sommer, J. *et al.* (1987) Biochemistry **26**:6407.
9. Gloor, S. *et al.* (1986) Cell **47**:687.
10. McGrogan, M. *et al.* (1988) Bio/Technology **6**:172.

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