Recombinant Human TFPI-2

Catalog Number: 2545-PI

DESCRIPTION

Source	Mouse myeloma cell line, NS0-derived Asp23-Lys213, with a C-terminal 10-His tag Accession # P48307
N-terminal Sequence Analysis	Asp23
Predicted Molecular Mass	23 kDa

SPECIFICATIONS

SDS-PAGE	33 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 <5 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	Lyophilized from a 0.2 µm filtered solution in Tris, NaCl and Brij-35. 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 TFPI-2 (rhTFPI-2) (Catalog # 2545-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.
2. Prepare a curve of rhTFPI-2 (MW: 23,197 Da) in Assay Buffer. Make the following serial dilutions: 4000, 800, 400, 200, 100, 50, 25, 12.5, 6.25, and 1.25 nM.
3. Combine equal volumes of diluted Trypsin and rhTFPI-2 at each concentration of the curve. Include two controls containing equal volumes of Assay Buffer and diluted Trypsin without any rhTFPI-2.
4. Incubate mixtures at 37 °C for 15 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 incubated mixtures in a plate, and start the reaction by adding 50 µL of 20 µM Substrate.
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% inhibiting concentration (IC_{50}) for rhTFPI-2 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-Leu-OH (Bachem, Catalog # M-1975).

Final Assay Conditions

Per Well:

- Trypsin: 0.00125 µg
- rhTFPI-2: 200, 40, 20, 10, 5, 2.5, 1.25, 0.625, 0.3125, and 0.0625 nM.
- Substrate: 10 µM

PREPARATION AND STORAGE

Reconstitution	Reconstitute at 100 µg/mL in sterile 25 mM Tris and 150 mM NaCl, pH 7.5.
Shipping	The product is shipped at ambient temperature. 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 reconstitution.

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BACKGROUND

Human Tissue Factor Pathway Inhibitor 2 (TFPI-2), also known as placental protein 5 (PP5) and retinal pigment epithelial cell factor 1 (REF-1), is a secreted protein with a N-terminal acidic region, three Kunitz (K) domains (residues 36 to 86, 96 to 149 and 158 to 208) separated with by two linker regions, and a C-terminal basic region (1-3). Expression of TFPI-2 is down-regulated in several cancers, which may contribute to tumor progression in these cancers (4). The purified rhTFPI-2 ends at residue 213 and does not contain the last 22 residues (residues 214 to 235) in the C-terminal region. It inhibits the activity of Recombinant Human Coagulation Factor VII (Catalog # 2338-SE) in the presence of Recombinant Human Coagulation Factor III/Tissue Factor (Catalog # 2339-PA) .

References:

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