Recombinant Human TFPI
Catalog Number: 2974-PI

DESCRIPTION
Source
Mouse myeloma cell line, NS0-derived human TFPI protein
Asp29-Lys282, with a C-terminal 10-His tag
Accession # P10646

N-terminal Sequence Analysis
Asp29

Predicted Molecular Mass
31 kDa

SPECIFICATIONS
SDS-PAGE
41 kDa, reducing conditions

Activity
Measured by its ability to inhibit trypsin cleavage of a fluorogenic peptide substrate, Mca-RPKPVE-Nval-WRK(Dnp)-NH₂ (Catalog # ES002).
The IC₅₀ value is <0.75 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 NaH₂PO₄ and NaCl. See Certificate of Analysis for details.

Activity Assay Protocol
Materials
- Assay Buffer: 50 mM Tris, 10 mM CaCl₂, 150 mM NaCl, 0.05% (w/v) Brij-35, pH 7.5 (TCNB)
- Recombinant Human TFPI1 (rhTFPI1) (Catalog # 2974-PI)
- Trypsin (Sigma, Catalog # T-1426)
- Substrate: MCA-Arg-Pro-Lys-Pro-Val-Glu-NVAL-Trp-Arg-Lys(DNP)-NH₂ (Catalog # ES002), 2 mM stock in DMSO
- 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 rhTFPI1 (MW: 30,525 Da) in Assay Buffer. Make the following serial dilutions: 500, 100, 50, 30, 20, 10, 5, 2.5, and 0.833 nM.
3. Combine equal volumes of 0.25 µg/mL Trypsin and rhTFPI1 serial curve dilutions. Include two controls containing equal volumes of Assay Buffer and 0.25 µg/mL Trypsin.
4. Incubate reaction mixtures at 37 °C for 15 minutes.
5. Dilute incubated reaction mixtures 5-fold in Assay Buffer.
6. Dilute Substrate to 20 µM in Assay Buffer.
7. In a plate, load 50 µL of the diluted reaction mixtures to wells, 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 (IC₅₀) for rhTFPI1 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):

Specific Activity (pmol/min/µg) = \frac{Adjusted \ V_{max} \ \text{RFU/min} \ \times \ Conversion \ Factor^*}{\ \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
- rhTFPI1 curve: 25, 5, 2.5, 1.5, 1.0, 0.5, 0.25, 0.125, and 0.0417 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.

DATA
Enzyme Activity

Recombinant Human TFPI (Catalog # 2974-PI) inhibits trypsin cleavage of a fluorogenic peptide substrate, Mca-RPKPVE-Nval-WRK(Dnp)-NH2 (Catalog # ES002).

**BACKGROUND**

Human TFPI, also known as lipoprotein-associated coagulation inhibitor (LACI) and extrinsic pathway inhibitor (EPI), is a physiological inhibitor of extrinsic pathway of coagulation and has biological functions of anticoagulation and anti-inflammation (1). It is a secreted protein with a N-terminal acidic region, three Kunitz (K) domains separated with by two linker regions, and a C-terminal basic region (2). The first K domain (residues 54 to 104) inhibits coagulation factor VIIa complexed to tissue factor (TF). The second K domain (residues 125 to 175) inhibits factor Xa. The third K domain (residues 217 to 267) binds to heparin (3). The C-terminal basic region may have several functions. For example, it plays an important role in binding of TFPI to cell surfaces (2). The purified Recombinant Human TFPI ends at residue 282 and does not contain the last 20 residues (residues 283 to 302) 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:**