Recombinant Human
Coagulation Factor III/Tissue Factor
Catalog Number: 2339-PA

DESCRIPTION
Source Mouse myeloma cell line, NS0-derived
Gly34-Glu251, with a C-terminal 6-His tag
Accession # P13726

N-terminal Sequence Analysis Gly34
Predicted Molecular Mass 26 kDa

SPECIFICATIONS
SDS-PAGE 41 kDa and 43 kDa, reducing conditions
Activity Measured by its ability to activate Coagulation Factor VII in cleaving a fluorogenic peptide substrate Boc-VPR-AMC (Catalog # ES011). The AC50 is <4.0 μg/mL, 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 Tris and NaCl. See Certificate of Analysis for details.

Activity Assay Protocol

Materials
- Activation Buffer: 50 mM Tris, 10 mM CaCl2, 150 mM NaCl, 0.05% (w/v) Brij-35, pH 7.5 (TCNB)
- Assay Buffer: 50 mM Tris, 2.5 mM CaCl2, pH 8.5
- Recombinant Human Coagulation Factor III/Tissue Factor (rhTF) (Catalog # 2339-PA)
- Recombinant Human Coagulation Factor VII (rhF7) (Catalog # 2338-SE)
- Bacterial Thermolysin (Thermolysin) (Catalog # 3097-ZN)
- 1,10 Phenanthroline (Sigma, Catalog # A9891)
- Substrate: BOC-Val-Pro-Arg-AMC (R&D Systems, Catalog # ES011), 100 mM in DMSO
- F16 Black Maxisorp Plate (Nunc, Catalog # 475515)
- Fluorescent Plate Reader (Model: SpectraMax Gemini EM by Molecular Devices) or equivalent

Assay
1. Dilute rhF7 to 100 μg/mL in Activation Buffer containing 6.25 μg/mL Thermolysin.
2. Incubate at 37 °C for 30 minutes to activate rhF7.
3. Stop reaction by adding 1,10 phenanthroline to a final concentration of 10 mM in Activation Buffer.
4. Incubate for 5 minutes at room temperature.
5. Prepare a dilution curve of rhTF in Assay Buffer. Make serial dilutions of: 71.04, 35.52, 17.76, 8.88, 4.44, 2.22, 1.11, 0.555, 0.278, and 0.139 μg/mL.
6. Dilute Thermolysin-activated rhF7 to 32 μg/mL in Assay Buffer.
7. Load 25 μL of each dilution from the dilution curve to a plate. Include two controls containing 25 μL Assay Buffer.
8. Add 25 μL of 32 μg/mL Thermolysin-activated rhF7 to all the wells.
9. Incubate at 37 °C for 5 minutes.
10. Dilute Substrate to 200 μM in Assay Buffer.
11. Add 50 μL of 200 μM Substrate to all wells.
12. Read at excitation and emission wavelengths of 380 nm and 460 nm (top read), respectively, in kinetic mode for 5 minutes.
13. Derive the 50% activating concentration (AC50) of rhTF by plotting RFU/min (or specific activity) vs. concentration with 4-PL fitting.
14. The specific activity for rhF7 at each point may be determined using the following formula (if needed):

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

*Adjusted for Substrate Blank
**Derived using calibration standard 7-amino, 4-methyl coumarin (Sigma, Catalog # A-9891).

Final Assay Conditions
Per Well:
- rhF7: 0.800 μg
- rhTF: 17.76, 8.88, 4.44, 2.22, 1.11, 0.556, 0.278, 0.139, 0.0695, and 0.0348 μg/mL
- Substrate: 100 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.
Coagulation Factor III/Tissue Factor (TF), also known as thromboplastin and CD142, is an integral membrane protein found in a variety of cell types. It functions as a protein cofactor/receptor of Coagulation Factor VII, which is synthesized in the liver and circulated in the plasma (1). Upon binding of TF, the inactive factor VII is rapidly converted into activated VIIa. The resulting 1:1 complex of VIIa and TF initiates the coagulation pathway and has also important coagulation-independent functions such as angiogenesis (2). Synthesized as a 295 amino acid precursor, TF consists of a signal peptide (residues 1 to 32) and the mature chain (residues 33 to 295). As a type I membrane protein, it contains a transmembrane region (residues 252 to 274) and a cytoplasmic tail (residues 275 to 295) (3-6). The purified rhTF corresponds to the ectodomain (residues 34 to 251) and is potent in activating thermolysin-processed Recombinant Human Coagulation Factor VII (Catalog # 2338-SE) under the conditions described in Activity Assay Protocol.

References: