Recombinant Mouse Coagulation Factor VII

Catalog Number: 3305-SE

DESCRIPTION

Source	Chinese Hamster Ovary cell line, CHO-derived Ala42-Leu446, with a C-terminal 10-His tag Accession # P70375
N-terminal Sequence Analysis	Ala42
Structure / Form	Mature form
Predicted Molecular Mass	47 kDa

SPECIFICATIONS

SDS-PAGE	58 kDa and 54 kDa, reducing conditions
Activity	Measured by its ability to cleave the fluorogenic peptide substrate Boc-VPR-AMC (Catalog # ES011). The specific activity, is >4 pmol/min/µg, 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 and NaCl. See Certificate of Analysis for details.

Activity Assay Protocol

Materials

- Activation Buffer: 50 mM Tris, 10 mM $CaCl_2$, 150 mM NaCl, 0.05% (w/v) Brij-35, pH 7.5 (TCNB)
- Assay Buffer: 50 mM Tris, pH 9.0
- Recombinant Mouse Coagulation Factor VII (rmFactor VII) (Catalog # 3305-SE)
- Bacterial Thermolysin (Thermolysin) (Catalog # 3097-ZN)
- 1,10-Phenanthroline (Sigma, Catalog # 320056)
- Recombinant Mouse Coagulation Factor III/Tissue Factor (rmTF) (Catalog # 3178-PA)
- Substrate: Boc-Val-Pro-Arg-AMC (Catalog # ES011), 10 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. Activate rmFactor VII at 100 µg/mL with 10 µg/mL Thermolysin in Activation Buffer.
2. Incubate at 37 °C for 30 minutes.
3. After incubation, stop reaction with 1,10-Phenanthroline at a final concentration of 10 mM in Activation Buffer.
4. Incubate reaction mixtures at 37 °C for 5 minutes. The enzyme concentration is now at 75 µg/mL.
5. Dilute rmTF to 15.3 µg/mL in Assay Buffer.
6. In a plate, load 13.3 µL of 75 µg/mL activated rmFactor VII followed by adding 36.7 µL of 15.3 µg/mL rmTF.
7. Incubate plate at 37 °C for 5 minutes.
8. Dilute Substrate to 200 µM in Assay Buffer.
9. After plate incubation, start the reaction by adding 50 µL of 200 µM of Substrate to wells.
10. Read at excitation and emission wavelengths of 380 nm and 460 nm (top read), respectively in kinetic mode for 5 minutes.
11. Calculate specific activity:

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

*Adjusted for Substrate Blank

**Derived using calibration standard 7-amino, 4-Methyl Coumarin (Sigma, Catalog # A-9891).

Final Assay Conditions

Per Well:

- rmFactor VII: 1 µg
- rmTF: 0.56 µg
- Substrate: 100 µM

PREPARATION AND STORAGE

Reconstitution	Reconstitute at 200 µg/mL in sterile 50 mM Tris, 10 mM $CaCl_2$, 150 mM NaCl and 0.05% Brij-35 (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

Coagulation Factors VII and VIIa refer to the pro and active forms of the same protease, respectively (1). Factor VII is synthesized in the liver and circulates in the plasma where it binds to tissue factor (TF), an integral membrane protein found in a variety of cell types. Upon binding of TF, factor VII is rapidly converted into VIIa. The resulting 1:1 complex of VIIa and TF initiates the coagulation pathway and has also important coagulation-independent functions such as angiognesis (2). The cleavage and activation of Coagulation Factors VII, IX and X by VIIa:TF is phospholipid-dependent whereas the cleavage of small peptide substrates is not (1). The deduced amino acid sequence of mouse factor VII predicts a signal peptide (residues 1 to 24), propeptide (residues 25 to 41), and the mature chain that can be further processed into the light chain (residues 42 to 193) and the heavy chain (residues 194 to 446). The purified recombinant mouse F7 corresponds to the mature chain, which can be processed and activated by treatment with thermolysin and binding with recombinant mouse Tissue Factor (Catalog # 3178-PA) under the conditions described in the Activity Assay Protocol.

References:

1. Morrissey, J.H. (2004) in *Handbook of Proteolytic Enzymes*, Barrett, A.J. *et al.* (eds.), Academic Press, San Diego, p. 1659.
2. Versteeg, H.H. *et al.* (2003) Carcinogenesis **24**:1009.

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