Recombinant Mouse Coagulation Factor XIV/Protein C

Catalog Number: 4885-SE

DESCRIPTION

Source	Chinese Hamster Ovary cell line, CHO-derived Ile19-Leu460 (pro) & Ala42-Leu460 (mature), both with a C-terminal 10-His tag Accession # P33587
N-terminal Sequence Analysis	Ile19 & Ala42
Structure / Form	Pro and Mature forms
Predicted Molecular Mass	49 kDa (Pro) & 48 kDa (Mature)

SPECIFICATIONS

SDS-PAGE	70 kDa, reducing conditions
Activity	Measured by its ability to cleave the fluorogenic peptide substrate, Boc-β-benzyl-DPR-AMC. The specific activity is >125 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	>90%, by SDS-PAGE under reducing conditions and visualized by silver stain.
Formulation	Supplied as a 0.2 µm filtered solution in Sodium Acetate 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, 10 mM $CaCl_2$, 150 mM NaCl, 0.01% (w/v) Brij-35, pH 8.5
- Recombinant Mouse Coagulation Factor XIV/Protein C (rmPROC) (Catalog # 4885-SE)
- Bacterial Thermolysin (Thermolysin) (Catalog # 3097-ZN)
- 1,10 phenanthroline (Sigma, Catalog # 320056), 0.6 M stock in DMSO
- Substrate: BOC-β-benzyl-Asp-Pro-Arg-AMC (Bachem, Catalog # I-1560), 10 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 rmPROC to 200 µg/mL in Activation Buffer.
2. Dilute Thermolysin to 10 µg/mL in Activation Buffer.
3. Combine 25 µL of the diluted rmPROC and 25 µL of the diluted Thermolysin.
4. Incubate at 37 °C for 2 hours.
5. Dilute 1,10 phenanthroline to 5 mM in Assay Buffer.
6. Add 200 µL of 5 mM 1,10 phenanthroline to the reaction mixtures to stop the Thermolysin activation.
7. Dilute rmPROC to 2 µg/mL in Assay Buffer.
8. Dilute Substrate to 200 µM in Assay Buffer.
9. Load 50 µL of 2 µg/mL rmPROC into a plate, and start the reaction by adding 50 µL of 200 µM Substrate.
10. Include a Substrate Blank containing 50 µL Assay Buffer and 50 µL of 200 µM Substrate.
11. Read at excitation and emission wavelengths of 380 nm and 460 nm (top read), respectively, in kinetic mode for 5 minutes.
12. 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 (AMC) (Sigma, Catalog # A-9891).

Final Assay Conditions

Per Well:

- rmPROC: 0.100 µg
- 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.

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Recombinant Mouse Coagulation Factor XIV/Protein C

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BACKGROUND

Protein C is a vitamin K-dependent serine protease synthesized in the liver as a single-chain precursor, which is then proteolytically processed to two disulfide-linked chains (1). The light chain consists of a Gla (gamma-carboxy-glutamate) domain and two EGF-like domains. The heavy chain consists of an activation peptide (aa 199-212) and serine protease domain (aa 213-449). Physiologically, Protein C is converted to the active form by thrombin, which releases the activation peptide. Protein C plays a key role in anticoagulation, cleaving factors VIIIa and Va to inactivate them. This anticoagulation activity can be enhanced by a presence of a cofactor such as protein S. In hereditary thrombophilia, Protein C deficiency is caused by a genetic mutation that affects Protein C activity. A severe recessive form may result in massive thrombosis fatal to patient.

References:

1. Shen, L. and Dahlbäck, B. (2004) in *Handbook of Proteolytic Enzymes*, Barrett, A.J. *et al*. eds. pp. 1673.

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