Recombinant Human Coagulation Factor XIV/Protein C

Catalog Number: 3349-SE

DESCRIPTION

Source	Mouse myeloma cell line, NS0-derived human Coagulation Factor XIV/Protein C protein Met1-Pro461, with a C-terminal 10-His tag Accession # P04070
N-terminal Sequence Analysis	Ala43 (mature and light chains) & Asp200 (heavy chain)
Predicted Molecular Mass	49 kDa (mature), 31 kDa (heavy), 18 kDa (light)

SPECIFICATIONS

SDS-PAGE	60-64 kDa, 41-44 kDa and 22 kDa, reducing conditions
Activity	Measured by its ability to cleave the fluorogenic peptide substrate Boc-VPR-AMC (Catalog # ES011). The specific activity is >25 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	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, 150 mM NaCl, 10 mM $CaCl_2$, 0.05% (w/v) Brij-35, pH 7.5 (TCNB)
- Assay Buffer: 50 mM Tris, 100 mM NaCl, 0.01% (w/v) Brij-35, pH 8.5
- Recombinant Human Coagulation Factor XIV/Protein C (rhPROC) (Catalog # 3349-SE)
- Bacterial Thermolysin (Thermolysin) (Catalog # 3097-ZN)
- 1,10-Phenanthroline (Sigma, Catalog # 320056), 0.6 M stock in DMSO
- Fluorogenic Peptide Substrate: BOC-Val-Pro-Arg-AMC (Catalog # ES011)
- F16 Black Maxisorp Plate (Nunc, Catalog # 475515)
- Fluorescent Plate Reader (Model: SpectraMax Gemini EM by Molecular Devices) or equivalent

Assay

1. Dilute rhPROC to 100 µg/mL in Activation Buffer with 2.3 µg/mL Thermolysin.
2. Incubate for 30 minutes at 37 °C.
3. Stop Thermolysin activity by adding 1,10-Phenanthroline to 4 mM.
4. Incubate for 15 minutes at room temperature.
5. Dilute rhPROC to 4 ng/µL in Assay Buffer.
6. Dilute Substrate to 200 µM in Assay Buffer.
7. Load into a black well plate 50 µL of 4 ng/µL rhPROC, and start the reaction by adding 50 µL of 200 µM Substrate. Include a Substrate Blank containing 50 µL of Assay buffer and 50 µL of 200 µM Substrate.
8. Read at excitation and emission wavelengths of 380 nm and 460 nm (top read), respectively, in kinetic mode for 5 minutes.
9. 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:

- rhPROC: 0.200 µg
- Substrate: 100 µM

PREPARATION AND STORAGE

Shipping	The product is shipped with dry ice or equivalent. 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.

bio-techne®
Global bio-techne.com info@bio-techne.com techsupport@bio-techne.com TEL +1 612 379 2956
USA TEL 800 343 7475 Canada TEL 855 668 8722 China TEL +86 (21) 52380373
Europe | Middle East | Africa TEL +44 (0)1235 529449

Recombinant Human Coagulation Factor XIV/Protein C

Catalog Number: 3349-SE

BACKGROUND

Protein C, also known as Coagulation Factor XIV, is a vitamin K-dependent serine protease synthesized in the liver as a single-chain precursor (1). The N-terminus consists of a signal peptide (amino acid (aa) 1-32) and a propeptide (aa 33-42). The mature chain (aa 43-461) is converted to two disulfide-linked chains (light: aa 43-199 and heavy: 200-461) and both forms are inactive. The light chain consists of Gla (gamma-carboxy-glutamate) domain and two EGF-like domains. The heavy chain consists of an activation peptide (aa 200-211) and a serine protease domain (aa 212-450). Present in plasma at 3 to 5 mg/L, protein C plays a key role in anticoagulation. Physiologically, the inactive forms of protein C are converted to the active form by thrombin, which releases the activation peptide. The active protein C cleaves factor 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 which affect protein C activity. A severe recessive form may result in a massive thrombosis, which is fatal to the patient. The recombinant human Protein C consists of both the mature chain and the two disulfide-linked chains, which can be activated by treatment with thermolysin.

References:

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

bio-techne®
Global bio-techne.com info@bio-techne.com techsupport@bio-techne.com TEL +1 612 379 2956
USA TEL 800 343 7475 **Canada** TEL 855 668 8722 **China** TEL +86 (21) 52380373
Europe | Middle East | Africa TEL +44 (0)1235 529449