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Recombinant Human Coagulation Factor X Animal Component Free

Catalog Number: ACFP1063

DESCRIPTION

Source	*Spodoptera frugiperda*, *Sf* 9 (baculovirus)-derived Met1-Lys488, with a C-terminal 10-His tag Accession # P00742 The pro form was activated and further purified. Produced in an animal component free process (ACFP).
N-terminal Sequence Analysis	Phe80, Tyr84, & Ile235
Structure / Form	Active Form. Recombinant Human Coagulation Factor X Animal Component Free is prone to proteolytic cleavage at C-terminus. The predominant form of the purified protein lacks the His tag.
Predicted Molecular Mass	16.6 kDa & 30 kDa

SPECIFICATIONS

SDS-PAGE	12-15 kDa and 30-36 kDa, reducing conditions
Activity	Measured by its ability to cleave the fluorogenic peptide substrate, Mca-RPKPVE-Nval-WRK(Dnp)-NH_2 (Catalog # ES002). The specific activity is >800 pmol/min/µg, as measured under the described conditions. See Activity Assay Protocol on www.RnDSystems.com
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 MES, NaCl and $CaCl_2$. See Certificate of Analysis for details.

Activity Assay Protocol

Materials

- Assay Buffer: 50 mM Tris, 10 mM $CaCl_2$, 150 mM NaCl, 0.05% Brij-35, pH 7.5 (TCNB)
- Recombinant Human Coagulation Factor X Animal Component Free (rhFactor X) (Catalog # ACFP1063)
- Substrate: Fluorogenic Peptide Substrate II, Mca-Arg-Pro-Lys-Pro-Val-Glu-Nval-Trp-Arg-Lys(Dnp)-NH_2 (Catalog # ES002)
- F16 Black Maxisorp Plate (Nunc, Catalog # 475515)
- Fluorescent Plate Reader (Model: SpectraMax Gemini EM by Molecular Devices) or equivalent

Assay

1. Dilute rhFactor X to 0.4 ng/µL in Assay Buffer.
2. Dilute Substrate to 20 µM in Assay Buffer.
3. Load 50 µL of the 0.4 ng/µL rhFactor X into plate and start the reaction by adding 50 µL of 20 µM Substrate. Include a Substrate Blank containing 50 µL Assay Buffer and 50 µL of 20 µM Substrate without any rhFactor X.
4. Read at excitation and emission wavelengths of 320 nm and 405 nm (top read), respectively, in kinetic mode for 5 minutes.
5. 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 MCA-Pro-Leu-OH (Bachem, Catalog # M-1975).

Final Assay Conditions Per Well

- rhFactor X: 0.02 µg
- Substrate: 10 µM

PREPARATION AND STORAGE

Reconstitution	Reconstitute at 100 µg/mL in sterile 25 mM MES, 150 mM NaCl and 5 mM $CaCl_2$, pH 6.0
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 reconstitution.

BACKGROUND

As the only known physiological activator of thrombin, Factor X is a vitamin K-dependent plasma protease that plays a pivotal role in blood coagulation. Human Factor X (rhFX) is initially synthesized in the liver as a single-chain precursor of 488 amino acid residues with a signal peptide and a pro region (residues 1-40). Both the intrinsic and extrinsic pathways activate Factor X to Xa, which consists of light (residues 41-179) and heavy (residues 235-488) chains linked by a disulfide bond. The light chain contains a Gla and two EGF-like domains and the heavy chain corresponds to the serine protease domain. The full-length human Factor X was expressed and the pro enzyme was purified and activated. The active protease was further purified and analyzed for its activity towards either peptides or proteins containing a Xa cleavage site. In addition to the activity described in the Activity Assay Protocol, rhFX also be used to cleave fusion proteins containing a Factor Xa cleavage site. The conditions for the optimal cleavage of a particular fusion protein, such as the molar ratio between rhFX and the protein and time and temperature of incubation, are protein-dependent and need to be individually determined.

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