

Background

Coagulation factors XI and XIa refer to the pro and active forms of the same protease, respectively (1). Factor XI is synthesized in the liver and circulates in the plasma as a disulfide bond-linked dimer complexed with high molecular weight kininogen. Factor XI is converted into XIa via either the contact phase of blood coagulation or thrombin-mediated activation on the platelet surface. The resulting XIa converts factor IX into IXa, which subsequently activates factor X into Xa. Factor Xa in turn activates factor II/thrombin to complete the coagulation cascade. Patients with factor XI deficiency are prone to excessive bleeding after hemostatic challenge. There are two alternative splicing forms. Isoform 1 corresponds to the circulating plasma factor XI and isoform 2 is produced by platelets and megakaryocytes but absent from other blood cells (2). The 624 amino acid precursor of isoform 1 consists of a signal peptide (residues 1 to 18) and the mature chain (residues 19 to 624). The mature chain (XI) can be further processed into the heavy chain (residues 19 to 389) and the light chain (residues 390 to 624) (XIa). The purified rmFactor XI corresponds to isoform 1 (residues 19 to 624), which can be activated by treatment with thermolysin under the conditions described in the Activity Assay Protocol.

References:

1. Wash, P.N. (2004) in *Handbook of Proteolytic Enzymes*, Barrett, A.J. et al, eds. p. 1651.
2. Hsu, T.C. et al. (1998) *J. Biol. Chem.* 273:13787.

Description

Source	Murine myeloma cell line, NS0-derived Glu19 - Val624, with a C-terminal 10-His tag Accession # NP_082342
N-terminal Sequence Analysis	Glu19
Structure / Form	Disulfide-linked homodimer
Predicted Molecular Mass	69 kDa

Specifications

SDS-PAGE	74 kDa, reducing conditions
Activity	Measured by its ability to cleave the fluorogenic peptide substrate, t-butylloxycarbonyl-Ile-Glu-Gly-Arg-7-amido-4-methylcoumarin (Boc-IEGR-AMC). The specific activity is > 100 pmoles/min/μg, as measured under the described conditions. See Activity Assay Protocol.
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 HEPES and NaCl. See Certificate of Analysis for details.

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. <ul style="list-style-type: none"> ● 6 months from date of receipt, -70 °C as supplied. ● 3 months, -70 °C under sterile conditions after opening.

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, 1 mM EDTA, pH 7.5
- Recombinant mouse Factor XI (R&D Systems, Catalog # 4556-SE)
- Thermolysin (R&D Systems, Catalog # 3097-ZN)
- EDTA, pH 8.0 (Sigma, Catalog # E-4884), 0.5 M stock in deionized water
- Substrate: Boc-Ile-Glu-Gly-Arg-AMC (Bachem, Catalog # I-1100), 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. Dilute rmFactor XI to 100 μg/mL in Activation Buffer containing 10 μg/mL of Thermolysin.
2. Incubate at 37 °C for 1 hour.
3. Stop reaction with EDTA at a final concentration of 40 mM.
4. Dilute incubated rmFactor XI to 2 ng/μL in Assay Buffer.
5. Dilute Substrate to 200 μM in Assay Buffer.
6. Load 50 μL of 2 ng/μL rmFactor XI into a plate, and start the reaction by adding 50 μL of 200 μM Substrate. Include a Substrate Blank containing 50 μL Assay Buffer and 50 μL of 200 μM Substrate.
7. Read at excitation and emission wavelengths of 380 nm and 460 nm (top read), respectively, in kinetic mode for 5 minutes.
8. Calculate specific activity:

$$\text{Specific Activity (pmoles/min/}\mu\text{g)} = \frac{\text{Adjusted } V_{\text{max}}^* \text{ (RFU/min)} \times \text{Conversion Factor}^{**} \text{ (pmole/RFU)}}{\text{amount of enzyme } (\mu\text{g)}}$$

*Adjusted for Substrate Blank

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

Final Assay Conditions Per Well

- rmFactor XI: 0.100 μg
- Thermolysin: 0.010 μg
- Substrate: 100 μM

12/21/2009

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NOT FOR USE IN HUMANS