Factor Xa

1-800-632-7799
info@neb.com
www.neb.com

P8010S 091120614061

P8010S

50 µg **Lot: 0911206** **Exp: 6/14**
1 mg/ml **Store at –20°C**

Recognition Site:

Ile-Glu/Asp-Gly-Arg▼

Description: Factor Xa cleaves after the arginine residue in its preferred cleavage site Ile-(Glu or Asp)-Gly-Arg. It will sometimes cleave at other basic residues, depending on the conformation of the protein substrate (1,2,3). The most common secondary site, among those that have been sequenced, is Gly-Arg. There seems to be a correlation between proteins that are unstable in *E. coli* and those that are cleaved by Factor Xa at secondary sites: this may indicate that these proteins are in a partially unfolded state (Walker, I., Riggs, P., unpublished observations). Factor Xa will not cleave a site followed by proline or arginine.

Source: Factor Xa protease is purified from bovine plasma and activated by treatment with the activating enzyme from Russell's viper venom.

Supplied in: 20 mM HEPES, 500 mM NaCl, 2 mM $CaCl_2$, 50% glycerol, (pH 8.0).

Molecular Weight: The predominant form of Factor Xa in our preparation has a molecular weight of approximately 43 kDa, consisting of two disulfide-linked chains of approximately 27 kDa and 16 kDa. On SDS-PAGE, the reduced chains have apparent molecular weights of 30 kDa and 20 kDa.

Inactivation: Dansyl-Glu-Gly-Arg-chloromethyl ketone (CALBIOCHEM, #251700) will irreversibly inactivate Factor Xa by covalent attachment at the active site. In a reaction containing 20 µg/ml Factor Xa, 2 µM dansyl-Glu-Gly-Arg-chloromethyl ketone will inactivate > 95% of the Factor Xa in 1 minute at room temperature.

Unit Definition: 1 µg of Factor Xa will cleave 50 µg of test substrate to 95% completion in 6 hours or less.

Unit Assay Conditions: 1 µg of Factor Xa is added to 50 µg of an MBP fusion protein test substrate, MBP-ΔSal. The reaction is carried out in 50 µl, 20 mM Tris-HCl, 100 mM NaCl, 2 mM $CaCl_2$, (pH 8.0). Incubate at 23°C.

The test substrate MBP-ΔSal is maltose-binding protein fused to a truncated form of paramyosin, with the amino acids Ile-Glu-Gly-Arg at the fusion joint. Greater than 95% of the fusion protein is cleaved in 6 hours or less.

Specificity: The products of the reaction described above were run on three identical SDS-polyacrylamide gels. One gel was stained with Coomassie brilliant blue, and the other two were blotted to nitrocellulose and the protein bands detected with anti-maltose binding protein or anti-paramyosin antibodies. Only two proteins bands were produced by the reaction.

The bands were identified as maltose binding protein and the paramyosin fragment by apparent molecular weight and by immunological staining.

References:

1. Nagai, K. et al. (1984) *PNAS USA* 82, 7252–7255.
2. Quinlan, R. A. et al. (1989) *J. Cell Sci.* 93, 71–83.
3. Eaton, D. et al. (1986) *Biochem.* 25, 505–512.

Activity is inhibited by concentrations above 250mM NaCl or Imidazole TX-100 less than 1%

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