



NOTICE: ProZyme was purchased by Agilent in July 2018. Documents for products and product lots manufactured before August 2019 will contain references to ProZyme. For more information about these products and support, go to: www.agilent.com/en/contact-us.



FACTOR Xa (lyophilized)

SPECIFICATIONS

Product Code: CP13

Specific Activity: >125 U/ mg

Formulation: When reconstituted as directed, contains 5 mM MES, 0.5 M NaCl, 5 mM Benzamidine (pH 6.5).

Shipped with cold pack for next day delivery.

Store at -20 °C. After reconstitution, store the Factor Xa at -70 °C.

Stability: The lyophilized enzyme is stable for at least a year when stored desiccated at -20 °C.

ProZyme Factor Xa is a serine protease isolated from bovine plasma. It is the activated form of factor X, a circulating zymogen. Factor Xa is derived from factor X in vivo by the action of components of either the intrinsic or extrinsic coagulation pathways. The physiological substrate for factor Xa is prothrombin but the rate of cleavage of prothrombin to thrombin is greatly enhanced by the inclusion of Ca⁺⁺, phospholipid and factor V in the reaction. The biochemistry of factor X has been reviewed by Steinberg and Nemerson (1982).

In addition to its use as a reagent for coagulation studies, factor Xa has been exploited as a tool for specific cleavage of

recombinant fusion proteins. Bovine factor Xa is known to recognize the sequences Ile-Glu-Gly-Arg, Ile-Asp-Gly-Arg and Ala-Glu-Gly-Arg (Nagai and Thøgersen, 1984).

CHARACTERISTICS

Molecular weight: 44,000 daltons

Composition: Factor X is a heterodimer. It is composed of 2 chains joined by disulfide bonds. The heavy chain has a molecular weight of 38,000 daltons while the light chain has a molecular weight of 16,000 daltons. During activation by Russell's viper venom, the heavy chain is cleaved, resulting in two forms of factor Xa, αXa (MW 44,000), which predominates, and βXa (MW 40,000) (Steinberg and Nemerson, 1982).

Extinction coefficient: $E_{280}^{1\%} = 10$ (measured)

Optimum reaction conditions: Rates of prothrombin activation in the complex system including Ca⁺⁺, phospholipid and factor V are reviewed by Jackson (1982). Optimized conditions for factor Xa with a synthetic substrate are given by Aurell et al (1977), see below.

Isoelectric point: 4.3

ASSAY

The release of p-nitroaniline (pNA) is followed spectrophotometrically at 405 nm ($\epsilon_{405} = 10,500$). One unit of enzyme releases 1 μmole pNA/min at 37°C.

Reagents

Buffer: 0.05 M Tris-HCl, 5 mM CaCl_2 , 0.2 M NaCl (pH 8.3)

Enzyme dilution buffer: Buffer containing 1% serum albumin.

Substrate: Buffer containing 1.0 mM S-2222 (Chromagenix, 1-800-526-5224)

Enzyme: Dilute enzyme to about 1 $\mu\text{g}/\text{ml}$ in enzyme dilution buffer. Enzyme may not be stored diluted.

Procedure

Adjust spectrophotometer to 405 nm and 37°C.

Pipette 0.8 ml substrate into a cuvette.

Pipette 0.15 ml buffer into the cuvette and allow to equilibrate to 37°C.

Pipette 0.05 ml diluted enzyme into the cuvette, mix, and record the change in A_{405} for 4–5 minutes.

Calculation

$$\text{Units/mg} = \frac{(\Delta A_{405}/\text{min})(V_R)}{(\text{mg enzyme per assay})(\epsilon_{405})}$$

where:

ΔA_{405} = change in absorbance at 405 nm

V_R = reaction volume (liters)

ϵ_{405} = micromolar extinction coefficient of p-nitroaniline (0.0105 liters μmole^{-1})

SUGGESTIONS FOR USE

ProZyme Factor Xa is provided in 5 mM benzamidine to prevent autodigestion during shipment and storage. If the protein concentration is to be determined by A_{280} , the enzyme must be dialyzed to remove interfering absorbance.

For cleavage of fusion proteins, ProZyme recommends the following conditions: ~1% mass ratio of Factor Xa to substrate in 50 mM Tris-HCl, 100 mM NaCl, 1 mM CaCl_2 (pH 8.0), 4–37°C, 2 hrs–overnight. These conditions are suggested as a starting point. Any given fusion protein may require modification of these conditions for optimal cleavage. Ca^{++} is not strictly required for activity, and may be omitted if necessary. However, addition of Ca^{++} appears to stabilize the Factor Xa.

During extended incubations, cleavage at sites other than the tetrapeptide recognition sequence may be observed. Digestion should be monitored to determine appropriate ratios of Factor Xa to substrate, as well as appropriate incubation times. Alternatively, Wearne (1990) has shown that a reversible modification of lysine residues in the fusion protein can limit non-specific proteolysis.

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