For life science research only.
Not for use in diagnostic procedures.

Restriction protease factor Xa

From bovine plasma

Cat. No. 11 585 924 001 3 × 100 µg
Cat. No. 11 179 896 001 3 × 250 µg

Version 20
Content version: June 2019

Store at +2 to +8°C

Content
Lyophilisate.

Specificity
The coagulation factor Xa is a serine protease which recognizes the amino acid sequence

– Ile – Glu – Gly – Arg ↓

with a high degree of specificity. The cleavage of this sequence activates the natural substrate prothrombin to thrombin.

However, this sequence may also be used as a restriction cleavage site for processing of recombinant fusion proteins (1). When unspecifity is caused by steric hindrance the introduction of an additional sequence can overcome the problem (4).

Purity
The restriction protease factor Xa does not contain any bovine serum albumin as a stabiliser. It is present in a highly purified form. Purity control by SDS polyacrylamide gelelectrophoresis ensures a constant quality from lot to lot. Specificity is tested for each production batch. The composition of the lyophilisate does not influence the cleavage of the fusion proteins.

Note
The contents of a vial may be used for several simultaneous digests. Repeating the digest a new vial should be used. In doing so, reproducibility can be guaranteed and possible contamination can be prevented.

Storage and Stability
Stable at +2 to +8°C, stored dry. A solution in redist. water may be used for up to 1 week when stored at +2 to +8°C.

Application

The contents of one vial of restriction protease factor Xa is dissolved in redist. water to a final protein concentration of 1 mg/ml. The proteins to be cleaved are dissolved in 100 mM NaCl, 50 mM Tris-HCl, 1 mM $CaCl_2$, pH 8.0. To increase the solubility of the substrate, urea or acetonitrile can be added up to a final concentration of 1.0 M and 10% (v/v), respectively, without significant inhibition of the enzyme activity ($< 10\%$).

The recommended amount of enzyme is 1/200 to 1/10 of the substrate by weight. Incubation should be carried out at +4°C to +25°C for 2 – 18 h (1,2,3). The optimum cleavage conditions have to be determined for each fusion protein. The release of the desired protein or peptide from the fusion protein is influenced by the adjacent amino acid sequences at the cleavage site, the size of the two fused components, and the accessibility of the cleavage site.

Ordering Information

Application	Product	Cat. No.	Pack size
Buffers/ Stabilizers	Tris-HCl DTT	10 812 846 001 10 197 777 001 10 708 984 001 10 708 992 001 10 709 000 001	500 g 2 g 10 g 50 g 100 g
Preparation of β-Galactosidase	β-Galactosidase, marker enzyme	10 745 731 001	25 mg
Cleavage of β-Gal-fusion proteins	Restriction protease Enterokinase	11 334 115 001 11 351 311 001	3 × 30 µg 3 × 250 µg

Changes to previous version

Editorial changes.

References

1 Nagai, K. & Thogersen, H. Ch. (1984) *Nature* **309,** 810-812.
2 Nagai, K. & Thogersen, H. Ch. (1987) *Methods Enzymol.* **153,** 461-491.
3 Sieg, K. et al. (1989) Gene 75, 262-270.
4 Lauritzen, C. et al. (1991) *Protein Expression and Purification* **2**, 372-378.

* available from Roche Diagnostics

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