

# Factor Xa Protease (Ile-Glu-Gly-Arg)

Product Information Sheet  
# EP0504



## SUMMARY

shipped at -20 °C; store at -20 °C

For research use only

## Product Description and Application

- Highly active cleavage of N-Ile-Glu-Gly-Arg/-C sequence at the carboxyl end
- Creates N-terminal authentic recombinant protein if cloned into the NruI site of the pAX5+ vector

Factor Xa Protease is a specific serine proteinase with an extended substrate-binding region matching the Ile-Glu-Gly-Arg tetra peptide segment (Magnusson, S. *et al.*, 1975). This endoprotease is commonly used as a "restriction protease" (Nagai, K. and Thogersen, H.C. 1984 & 1987) to cleave recombinant fusion proteins specifically after the Arg residue at an inserted Ile-Glu-Gly-Arg junction (Sieg, K. *et al.*, 1989; Hiraoka, O. *et al.*, 1994; Schmidt, H.H. *et al.*, 1995; Nilson, B.H. *et al.*, 1996; Weller, U. *et al.*, 1996). Expression of desired proteins or polypeptides as fusion proteins in prokaryotes will routinely produce high yields with minimal expense and lab time investment. The inserted Ile-Glu-Gly-Arg segment in front of the protein of interest allows you to vary the handling properties of the fusion protein (i.e. charge, solubility, toxicity etc.) and to incorporate polypeptide segments conferring highly selective affinity for immobilized monoclonal antibodies or other suitable affinity substances to allow single step purifications. Following purification, the fusion protein is treated with Factor Xa Protease to release the desired protein with a precise N-terminal sequence determined by your vector construction. This strategy of recombinant protein production provides complete control over the N-terminal structure of the final product. Possible sequence heterogeneity due to variable extend of N-terminal processing at the methionyl residue arising from the translation initiation codon becomes unimportant, since the N-terminal part of the chimeric protein is removed *in vitro*. Factor Xa-cleavable fusion proteins are particular useful to prepare large amounts of authentic protein for structural work for any other application requiring substantial amounts of high quality recombinant protein products. With our Fusion Protein Vector pAX5+, an N-terminal authentic recombinant protein is released, provided the gene was cloned into the NruI site of the vector.

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

## Technical Details

<b>Systematic name:</b>	Factor Xa (EC 3.4.21.6)
<b>Source:</b>	Bovine plasma; activated by treatment with the activating enzyme from Russell's viper venom.
<b>Concentration:</b>	1 mg/ml (store solution at -20 °C)
<b>Storage buffer:</b> (pH)	Factor Xa protease Ile-Glu/Asp-Gly-Arg is supplied in 20 mM HEPES 8.0, 0.5 M NaCl, 2 mM CaCl <sub>2</sub> , 50% glycerol.
<b>Purity and activity checks:</b>	SDS-PAGE analysis and quantitative enzyme specificity analysis using an array of peptide substrates and recombinant Xa-cleavable fusion proteins.

# Factor Xa Protease (Ile-Glu-Gly-Arg)

Product Information Sheet  
# EP0504



## Buffer requirements for cleavage reaction:

pH optimum: 7.5 to 8.5; at pH 10 activity decreases significantly (to about 25%). Mercaptans should be below the millimolar level. 1% Nonidet P-40 and 0.5 M urea (but not 1 M!) are tolerated.

## Hints for optimization of the cleavage reaction:

The rate of specific cleavage by Factor Xa Protease is determined by the availability of the Ile-Glu-Gly-Arg tetra peptide site. It is influenced by neighbouring polypeptide segments. The reaction parameters should be varied to find conditions where your protein is properly in solution and where the Factor Xa Protease remains active for the time span required. For fusion proteins the reaction conditions have to be determined empirically. Typical parameters to be varied are:

- enzyme : substrate ratio: 1:10 to 1:200 (w/w)
- substrate concentration: 0.1 to 20 mg/ml
- temperature: 0 °C to 37 °C
- time: 1 to 20 hours
- pH: 7 to 10

## Unit definition:

One unit is the amount of Factor Xa Protease required to cleave 50 µg of test substrate to 95% completion in 6 hours or less.  
1 µg of Factor Xa is added to 50 µg of an MBP fusion protein test substrate, MBP-Sal. The assay is performed in 50 µl, 20 mM Tris (pH 8.0), 100 mM NaCl, 2 mM CaCl<sub>2</sub> at 23 °C, and the reaction is monitored by reading the absorbance at 405 nm.

## Removal & Inactivation:

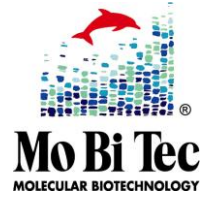
Factor Xa is irreversibly inactivated by Dansyl-Glu-Gly-Arg-chloromethyl ketone (Calbiochem, #251700). 2 µM of it will inactivate more than 95% of Factor Xa in a reaction (20 µg Factor Xa/ml) in 1 minute at room temperature. Factor Xa binds specifically to benzamidine-agarose (e.g. Sigma B-2768).

## References

- Hiraoka, O. *et al.*, *J. Biol. chem.* 269 (1994) 22412  
Magnusson, S. *et al.*, in `Proteases and Biological Control` Ed. Reich *et al.* (1975) p. CSH, NY  
Nagai, K. and Thogersen, H.C., *Nature* (London) 309 (1984) 810  
Nagai, K. and Thogersen, H.C., *Meth. Enzymol.* 153 (1987) 461  
Nilson, B.H. *et al.*, *Gene Ther.* 3 (1996) 280  
Schmidt, H.H. *et al.*, *J. Biol. Chem.* 270 (1995) 5469  
Sieg, K. *et al.*, *Gene* 75 (1989) 262  
Weller, U. *et al.*, *Eur. J. Biochem.* 236 (1996) 34

# Factor Xa Protease (Ile-Glu-Gly-Arg)

Product Information Sheet  
# EP0504



## Order Information, Shipping and Storage

Order#	Product	Quantity
EP0504	Factor Xa Protease (Ile-Glu-Gly-Arg)	250 µg
shipped at -20 °C; store at -20 °C		

Shipped at -20 °C; stored at -20 °C. Avoid multiple freeze-thaw cycles.

## Contact and Support

MoBiTec GmbH ● Lotzestrasse 22a ● D-37083 Goettingen ● Germany

### Customer Service – General inquiries & orders

phone: +49 (0)551 707 22 0  
fax: +49 (0)551 707 22 22  
e-mail: [order@mobitec.com](mailto:order@mobitec.com)

### Technical Service – Product information

phone: +49 (0)551 707 22 70  
fax: +49 (0)551 707 22 77  
e-mail: [info@mobitec.com](mailto:info@mobitec.com)

**MoBiTec in your area:** Find your local distributor at [www.mobitec.com](http://www.mobitec.com)