Factor Xa (Bovine)

32520 32521

<table>
<thead>
<tr>
<th>Number</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>32520</td>
<td>Factor Xa (Bovine), 250µg, supplied in 5mM MES (2-(N-morpholino) ethane sulfonic acid), 0.5M NaCl; pH 6.0, 1mM benzamidine•HCl</td>
</tr>
<tr>
<td>32521</td>
<td>Factor Xa (Bovine), 50µg, supplied in 5mM MES, 0.5M NaCl; pH 6.0, 1mM benzamidine•HCl</td>
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</tbody>
</table>

**Storage:** Upon receipt store product at -80°C. Product is shipped with dry ice.

**Introduction**

Thermo Scientific Factor Xa is an endoprotease formed by the activation of Factor X. The active site of Factor Xa is similar to the active site of trypsin. Factor Xa activity converts prothrombin to thrombin, a protein essential to blood-clotting. Factor Xa will cleave any peptide bond preceded by isoleucine-glutamic acid-glycine-arginine (Ile-Glu-Gly-Arg) or isoleucine-aspartic acid-glycine-arginine (Ile-Asp-Gly-Arg), unless proline occupies the $P'_1$ site, the site after the cleavage position.

The highly specific proteolytic activity of Factor Xa (Bovine) makes it useful for protein engineering. Many eukaryotic genes are difficult to overexpress in bacterial systems. One option is to fuse the eukaryotic sequences to prokaryotic genes. The genes are then expressed as a fusion protein in the bacterial system; however, the bacterial portion must be removed to use the protein for research. Retrieving the eukaryotic protein from the fusion product can be difficult. The process can be facilitated by inserting specific peptide sequences between the hybridized proteins to provide recognition sites for proteolytic enzymes. The tetrapeptide recognition sequence for Factor Xa (Bovine) is rare in protein sequences and, therefore, offers excellent specificity with minimal risk of damaging the protein of interest by random or internal cleavage.

When constructing the fusion protein expression vector, oligonucleotides coding for the recognition sequence of Factor Xa must be inserted between the fusion genes. The ligated plasmid is then transformed into a competent host strain where the genes can be expressed. Appropriate selection techniques are used to determine which colonies carry the recombinant plasmid. Once a correct colony has been identified, it is cultured using conditions optimal for expression of the fusion product. The hybrid protein is then purified and digested with Factor Xa, releasing the eukaryotic portion.

**Important Product Information**

- To minimize activity loss, avoid repeated cycles of freezing and thawing of Factor Xa. For best results, store Factor Xa in single-use volumes.
- Two complementary oligonucleotides coding for the Factor Xa recognition sequence must be synthesized (Table 1). The Factor Xa recognition sequence and the eukaryotic gene must be inserted into the vector in the proper orientation and in the correct translational reading frame. Make sure the protein of interest does not contain a Factor Xa recognition sequence.
- In choosing the expression vector, consider variables that optimize transcription and translation, such as promoters, ribosome-binding sites (Shine-Dalgarno sequences) and genetic markers, to facilitate selection of vector carrying colonies or capable of producing plaques.
- In choosing the prokaryotic gene for the fusion, consider how well the protein is normally expressed in *E. coli*, protein toxicity to the host, and the presence of inducible genes that can aid in selecting positive colonies or plaques.
Activity of Factor Xa (Bovine)

The activity of Factor Xa (Bovine) is measured using the synthetic tetrapeptide S-2222 (Kabi Diagnostics, Franklin, OH). This tetrapeptide contains the specified amino acid sequence with p-nitroanilide attached to the arginine. Factor Xa (Bovine) hydrolyzes S-2222 to release free p-nitroaniline, which is measured at 405nm. The assay buffer contains 0.8mM S-2222 and 0.05µg/mL of Factor Xa in Tris-buffered saline (TBS, 50mM Tris, 200mM NaCl, 5mM CaCl2, pH 8.3). The assay is performed at 37°C and the change in absorbance at 405nm is recorded for 4-5 minutes. Our Factor Xa (Bovine) is free of extraneous activating proteins. Factor Xa has high purity and specific activity (> 125U/mg) and can therefore be used at higher dilution rates than other commercially available products.

### Table 1. Possible codons for the Ile-Glu-Gly-Arg sequence (5' – 3').

<table>
<thead>
<tr>
<th>Ile</th>
<th>Glu</th>
<th>Gly</th>
<th>Arg</th>
</tr>
</thead>
<tbody>
<tr>
<td>AAT</td>
<td>TTC</td>
<td>ACC</td>
<td>ACG</td>
</tr>
<tr>
<td>GAT</td>
<td>CTC</td>
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<tr>
<td>TAT</td>
<td>CCC</td>
<td>TCG</td>
<td>CCG</td>
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</tbody>
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Protocol for Cleaving Fusion Proteins using Factor Xa

The following protocol was adapted from Nagai and Thøgersen. In this protocol, the expression vector is used to fuse the gene encoding the 31 amino-terminal residues of the lcII protein to the gene for human β-globin. The oligonucleotide sequence coding for the Ile-Glu-Gly-Arg recognition sequence of Factor Xa was inserted between the fusion genes. This protocol may be optimized for specific applications.

1. Dissolve or dialyze the protein to be hydrolyzed in TBS buffer (50mM Tris, 100mM NaCl, 6mM CaCl2 pH 8.0).
2. Add the diluted Factor Xa to the protein solution at an enzyme to substrate mass ratio of 1:100.
   **Note:** Digestion time and enzyme-to-substrate ratio may require optimization for each specific application.
3. Digest at 25°C for at least 2 hours. The digestion time can be increased if necessary. The progress of the hydrolysis can be determined by removing aliquots from the digestion mixture and performing polyacrylamide gel electrophoresis.
   **Note:** During extended incubation, cleavage at sites other than the peptide recognition sequence may be observed due to protease contamination of the fusion protein.
4. The hydrolyzed proteins can be analyzed on a polyacrylamide-SDS gel.

General References


This product (“Product”) is warranted to operate or perform substantially in conformance with published Product specifications in effect at the time of sale, as set forth in the Product documentation, specifications and/or accompanying package inserts (“Documentation”) and to be free from defects in material and workmanship. Unless otherwise expressly authorized in writing, Products are supplied for research use only. No claim of suitability for use in applications regulated by FDA is made. The warranty provided herein is valid only when used by properly trained individuals. Unless otherwise stated in the Documentation, this warranty is limited to one year from date of shipment when the Product is subjected to normal, proper and intended usage. This warranty does not extend to anyone other than the original purchaser of the Product (“Buyer”).

No other warranties, express or implied, are granted, including without limitation, implied warranties of merchantability, fitness for any particular purpose, or non infringement. Buyer’s exclusive remedy for non-conforming Products during the warranty period is limited to replacement of or refund for the non-conforming Product(s).

There is no obligation to replace Products as the result of (i) accident, disaster or event of force majeure, (ii) misuse, fault or negligence of or by Buyer, (iii) use of the Products in a manner for which they were not designed, or (iv) improper storage and handling of the Products.

Current product instructions are available at [www.thermoscientific.com/pierce](http://www.thermoscientific.com/pierce). For a faxed copy, call 800-874-3723 or contact your local distributor.

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