

Product Data Sheet

Product Name: Factor Xa Protease

Cat. No.: GC26287

Chemical Properties

Cas. No.

Formula

M.Wt 43 kDa

Solubility

Storage Store at -20°C

General tips For obtaining a higher solubility, please warm the tube at 37 °C and shake it in the ultrasonic bath for a while. Stock solution can be stored below -20°C for several months.

Shipping Condition Evaluation sample solution : ship with blue ice All other available size: ship with RT, or blue ice upon request.

Structure



Protocol

If necessary, concentrate the fusion protein to at least 1 mg/ml.

Caution: Product has not been fully validated for medical applications. For research use only.

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Do a pilot experiment with a small portion of your protein.

Example: Mix 20 μ l fusion protein at 1 mg/ml, with 1 μ l Factor Xa diluted to 200 μ g/ml, or 0.2 ng Enterokinase

In a separate tube, place 5 μ l fusion protein with no protease (mock digestion). Incubate the tubes at room temperature. At 2, 4, 8, and 24 hours, take 5 μ l of the reaction, add 5 μ l 2x SDS-PAGE Sample Buffer, and save at 4°C. Prepare a sample of 5 μ l fusion protein + 5 μ l 2X sample buffer (uncut fusion).

Boil the 6 samples for 5 minutes and run on an SDS-PAGE gel.

Scale the pilot experiment up for the portion of the fusion protein to be cleaved. Save at least a small sample of the uncut fusion as a reference.

Check for complete cleavage by SDS-PAGE.

Denaturing the Fusion Protein

1. Either dialyze the fusion against at least 10 volumes 20 mM Tris-HCl, 6 M guanidine hydrochloride, pH 7.4 for 4 hours, or add guanidine hydrochloride directly to the sample to give a final concentration of 6 M.
2. Dialyze against 100 volumes Column Buffer, 2 times at 4 hours each.

During refolding, one has to balance between two objectives. For the protease to cleave it must be present before the protein has completely refolded, so removing the denaturant quickly is desirable. However, when the denaturant is removed quickly some proteins will fail to refold properly and precipitate. Stepwise dialysis against buffer containing decreasing amounts of guanidine hydrochloride can prevent precipitation of the fusion protein; halving the guanidine concentration at

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each step is convenient, but cases where 0.1 M steps are necessary have been reported. However, if the fusion protein is able to refold into a protease-resistant conformation, it may be better to dialyze away the denaturant in one step and take the loss from precipitation in order to maximize the amount of cleavable fusion protein recovered.

Go to step 2 or 4 above, as appropriate.

Background

For Factor Xa, fusion protein cleavage is carried out at a w/w ratio of 1% the amount of fusion protein (e.g., 1 mg Factor Xa for a reaction containing 100 mg fusion protein). The reaction mixture can be incubated for 3 hours to several days, at room temperature or 4°C. Depending on the particular fusion protein, the amount of protease can be adjusted within the range of 0.1–5.0%, to get an acceptable rate of cleavage. Factor Xa will cleave at non-canonical sites in some proteins; for some fusions, there is a correlation between instability of the protein of interest in and cleavage at additional sites (unpublished observations). Presumably this cleavage activity depends on the three dimensional conformation of the fusion protein. For fusions that are resistant to cleavage, two strategies can sometimes help. Inclusion of small amounts of SDS (0.005–0.05%) in the reaction appears to relax the fusion enough to allow for cleavage in some cases. The window of SDS concentrations that work can be small, so a pilot titration with different SDS concentrations is necessary. Another strategy that sometimes helps is to denature the fusion to render the protease site accessible to cleavage.

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