Factor Xa Protease Treatment of Fusion Proteins Containing a Factor Xa Protease Recognition Sequence: Factor Xa Protease Cleavage

Factor Xa Protease (cat. no. 33223) can be stored at –20°C for up to 6 months.

For more information, including information on vectors, buffers, and protein purification procedures, please refer to *The QIA*expressionist which can be found at www.qiagen.com/handbooks.

Treatment of fusion proteins containing a Factor Xa Protease recognition sequence consists of three steps. The first step is Factor Xa Protease cleavage. This is followed by removal of Factor Xa Protease, and cleanup of the digested protein (see *Quick-Start Protocol*: *Factor Xa Protease Treatment of Fusion Proteins Containing a Factor Xa Recognition Sequence: Removal of Factor Xa Protease*).

For technical assistance, please call toll-free 00800-22-44-6000, or find regional phone numbers at www.qiagen.com/contact.

Factor Xa Protease cleavage

Notes before starting

- Detailed recommendations for optimization of the Factor Xa cleavage reaction are available in *The QIA*expressionist.

1. Prepare four solutions of 10 µg of the protein to be cleaved in 1x reaction buffer. Protein concentration should be at least 0.25 µg/µl.

   We recommend changing the buffer system if you have purified your protein by Ni-NTA affinity chromatography under native conditions because Factor Xa activity is reduced in phosphate buffer.

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2. Prepare three serial dilutions of Factor Xa Protease in 1x reaction buffer with concentrations of 1.0, 0.2, and 0.05 Units per µl.

   Note: Diluted protease should be used immediately after preparation.

3. Add 1 µl of each Factor Xa Protease dilution to one solution of the protein to be cleaved, and adjust the reaction volume to 40 µl using 1x reaction buffer. Adjust the volume of the fourth protein solution to 40 µl using 1x reaction buffer. This sample will serve as a negative control (see Table 1).

4. Incubate the reactions at room temperature (15–25°C). Take an 8 µl aliquot from each reaction after 3, 6, 9, and 16 h. Add 2 µl 5x SDS-PAGE sample buffer to each aliquot and mix thoroughly.

   Note: Mix aliquots with SDS-PAGE sample buffer immediately to completely quench Factor Xa Protease activity.

5. Analyze the efficiency of cleavage in each sample by SDS-PAGE.

   Note: We recommend using a gradient gel that will give good resolution in the size-range of the protein being analyzed.

   Note: Once optimal cleavage conditions have been found, the reaction can be scaled up. Following this protocol, 1 mg would be digested in 4 ml.

Table 1. Setup for optimization of Factor Xa Protease cleavage

Component	Volume/reaction	Final concentration
10 µg fusion protein	Variable	0.25 µg/µl
Factor Xa Protease dilutions (step 2)	1 µl	1.0, 0.2, and 0.05 U/40 µl
1x reaction buffer	Adjust to 40 µl	–