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

Xa Removal Resin (cat. no. 33213) can be stored at 2–8°C for up to 6 months. Xa Removal Resin should not be frozen.

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 (see *Quick-Start Protocol*: *Factor Xa Protease Treatment of Fusion Proteins Containing a Factor Xa Recognition Sequence: Factor Xa Protease Cleavage*). This is followed by removal of Factor Xa Protease, and cleanup of the digested protein.

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

Removal of Factor Xa Protease

1. Calculate the required amount of Xa Removal Resin necessary to capture the Factor Xa Protease present in the cleavage reaction.

   Note: A 50 µl bed volume (100 µl slurry) is sufficient to bind 4 Units Factor Xa Protease enzyme in 1x reaction buffer.

2. Resuspend the Xa Removal Resin completely by gentle inversion and then immediately transfer the required amount of slurry into a centrifuge tube.

3. Centrifuge the beads for 5 min at 1000 x *g* and discard the supernatant.

4. Resuspend the beads in ten bed-volumes of 1x reaction buffer by gently mixing, centrifuge for 5 min at 1000 x *g*, and discard the supernatant. Use Xa Removal Resin immediately after equilibration.

January 2011

5. Add the cleavage reaction to the equilibrated resin. Mix gently and incubate for 10 min at room temperature with gentle shaking. Binding can be performed at 4°C without any loss of binding efficiency.
6. Centrifuge the reaction at 1000 x *g* for 5 min to pellet the resin. Collect the supernatant which contains the cleaved protein. Factor Xa Protease remains bound to the resin.

Removal of cleaved 6xHis peptides after Factor Xa Protease cleavage

Notes before starting

- The protocol can be carried out either directly after Factor Xa Protease cleavage or after the removal of the protease with Xa Removal Resin.

1. Adjust the pH of the cleavage reaction mixture to 7.5. It is not necessary to adjust the pH if the protease digestion was performed at pH 7.5.
2. Calculate the required amount of Ni-NTA Agarose needed to capture the 6xHis-tagged contaminants. A 1 ml bed volume (2 ml slurry) is sufficient to bind 20–50 mg 6xHis-tagged protein.
3. Resuspend the Ni-NTA Agarose by inverting the bottle 4–6 times. Transfer the required amount of slurry into a centrifuge tube of appropriate size.
4. Centrifuge the resin for 1 min at 1000 x *g* and discard the supernatant.
5. Transfer the reaction mixture to the equilibrated resin. Mix gently to resuspend the resin and incubate for 10 min at room temperature with gentle shaking. Binding can be performed at 4°C without any loss of binding efficiency.
6. Centrifuge the suspension at 1000 x *g* for 1 min. Collect the supernatant that contains the pure recombinant protein.