Thrombin cleavage

(using thrombin produced by Amersham)

1. Thrombin cleavage of eluted fusion protein bound to Sepharose

* Mix 50 µl of thrombin (< 10 cleavage U/ml) solution and 950 µl of 1 x PBS for each ml of Glutathione Sepharose bed volume

1. Add thrombin protease mixture to Glutathione Sepharose pellet
2. Gently shake or rotate the suspension at r.t. for 2-16 h
3. Centrifuge the suspension at 500 x g for 5' to pellet the beads and carefully transfer the eluted fraction to a clean tube.

2. Thrombin cleavage of eluted fusion protein.

1. Add 10 µl of thrombin solution (10 cleavage units) per mg fusion protein. If the amount of fusion protein in the eluate has not been determined, add 80 µl (80 U) of thrombin for each ml of Glutathione Sepharose bed volume from with the fusion protein was eluted.
2. Mix gently and incubate at r.t. (22-25C) for 2-16 h.
3. Once digestion is complete, GST can be removed by first removing glutathione by extensive dialysis (2,000 vol/ml) against 1 x PBS followed by batch purification.