Separating the Protein of Interest from MBP after Protease Cleavage Using The pMAL Protein Fusion and Purification System (E8200)

Overview

Protocol

1. **Method I**

   Anion exchange chromatography

   This method potentially purifies the target protein away from MBP and the protease, but also provides an additional purification step for removing trace contaminants. A disadvantage is that occasionally the peak containing the protein of interest overlaps with MBP or the protease, resulting in poor separation. The procedure is written for quantities < 25 mg, and can be scaled up for larger amounts. The procedure calls for an anion exchange column such as the HiTrap Q FF (GE Life Sciences #17-5156-01).

   1. Dialyze the fusion protein cleavage mixture vs. 20 mM Tris-HCl, 25 mM NaCl, pH 8.0 (2 or 3 changes of 100 volumes, at least 2 hours each).

   2. Wash the column with 15 ml of the same buffer.

   3. Load the fusion protein cleavage mixture onto the column. Collect 2.5 ml fractions of the column flow-through.

   4. Wash the column with 3–5 column volumes of the same buffer. Continue collecting 2.5 ml fractions.

   5. Start a gradient of 25 mM NaCl to 500 mM NaCl (25 ml each) in 20 mM Tris-HCl, pH 8.0 (Figure 3). Collect 1 ml fractions.

   6. Determine which fractions contain protein by measuring A_{280}, or by the Bradford method. The MBP elutes as a sharp peak at 100–150 mM NaCl. Factor Xa elutes at about 400 mM NaCl. The target protein may flow through the column, or it may elute during the gradient. Electrophorese the relevant fractions on an SDS-PAGE gel (12). Pool the fractions containing the target protein free of MBP and concentrate as desired.

   If your protein is not separated from MBP using anion exchange chromatography, other chromatography resins can be tried. HiTrap SP (phosphate buffer at pH 7.0; MBP flows through) and gel filtration are good alternatives.

2. **Method II**

   Anion exchange chromatography variation

   This method takes advantage of the fact that MBP and Factor Xa will bind to a positively-charged resin at pH 5.5, where most other proteins do not bind. The procedure calls for an anion exchange column such as the HiTrap Q FF (GE Life Sciences #17-5156-01).

1. **Dialyze the cleavage mixture vs. 20 mM sodium phosphate buffer, 25 mM NaCl, pH 5.5** (2 or 3 changes of 100 volumes, at least 2 hours each)

2. Wash the column with 15 ml of the same buffer.

3. Load the fusion protein cleavage mixture onto the column. Collect 2.5 ml fractions of the column flow-through.

4. Wash the column with 3–5 column volumes of the same buffer. Continue collecting 2.5 ml fractions.

5. Check the flow-through fractions for protein by A280 or Bradford. In most cases, the protein of interest will flow through the column.