

## Removal of poly-histidine fusion tags from recombinant proteins purified by expanded bed adsorption

### ABSTRACT

Enzymatic methods have been used to cleave the C- or N-terminus polyhistidine tags from histidine tagged proteins following expanded bed purification using immobilized metal affinity chromatography (IMAC). This study assesses the use of Factor Xa and a genetically engineered exopeptidase dipeptidyl aminopeptidase-1 (DAPase-1) for the removal of C-terminus and N-terminus polyhistidine tags, respectively. Model proteins consisting of maltose binding protein (MBP) having a C- or N-terminal polyhistidine tag were used. Digestion of the hexahistidine tag of MBP-His6 by Factor Xa and HT 15-MBP by DAPase-1 was successful. The time taken to complete the conversion of MBP-His6 to MBP was 16 h, as judged by SDS-PAGE and Western blots against anti-His antibody. When the detagged protein was purified using subtractive IMAC, the yield was moderate at 71% although the overall recovery was high at 95%. Likewise, a yield of 79% and a recovery of 97% was obtained when digestion was performed with using "on-column" tag digestion. On-column tag digestion involves cleavage of histidine tag from polyhistidine tagged proteins that are still bound to the IMAC column. Digestion of an N-terminal polyhistidine tag from HT15-MBP (1 mg/mL) by the DAPase-1 system was superior to the results obtained with Factor Xa with a higher yield and recovery of 99% and 95%, respectively. The digestion by DAPase-1 system was faster and was complete at 5 h as opposed to 16 h for Factor Xa. The detagged MBP proteins were isolated from the digestion mixtures using a simple subtractive IMAC column procedure with the detagged protein appearing in the flowthrough and washing fractions while residual dipeptides and DAPase-1 (which was engineered to exhibit a poly-His tail) were adsorbed to the column. FPLC analysis using a MonoS cation exchanger was performed to understand and monitor the progress and time course of DAPase-1 digestion of HT15-MBP to MBP. Optimization of process variables such as temperature, protein concentration, and enzyme activity was developed for the DAPase-1 digesting system on HT15-MBP to MBP. In short, this study proved that the use of either Factor Xa or DAPase-1 for the digestion of polyhistidine tags is simple and efficient and can be carried out under mild reaction conditions. © 2005 Wiley Periodicals, Inc.

**Keyword:** DAPase; Expanded bed adsorption; Factor Xa; Immobilized metal affinity chromatography; Removal of polyhistidine tails