Purification of MBP-tagged proteins using new prepacked columns

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Purification of MBP-tagged proteins using new prepacked columns

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Background

Maltose binding protein (MBP) is beneficial as an affinity tag due to its ability to increase expression level and solubility of the fusion protein. The new MBPTrap™ HP 1 ml and 5 ml columns have been specially developed for high specificity towards the MBP-tag and the prepacked format facilitate fast and convenient purification. The small bead size of the medium, Dextrin Sepharose™ High Performance (34 µm), allows elution in narrow peaks minimizing the need for further concentration steps.

MBPTrap HP 1 ml and 5 ml columns quickly and conveniently purify MBP-tagged recombinant proteins to high purities in concentrated forms and small volumes.

MBPTrap HP characteristics

Column dimensions:	0.7 x 2.5 cm (1 ml) and 1.6 x 2.5 cm (5 ml)
Bed volume:	1 ml or 5 ml
Medium:	Dextrin Sepharose High Performance
Dynamic binding capacity:	Approx. 10 mg MBP-tagged protein/ml medium (protein dependent)
Recommended flow rate:	1 and 5 ml/min for 1 and 5 ml columns, respectively
Maximum flow rate:	4 and 20 ml/min for 1 and 5 ml columns, respectively
pH working range:	> pH 7
Storage:	4°C to 8°C in 20% ethanol

Conclusions

MBPTrap HP provides the following benefits:

- High final purity due to the specificity towards the MBP-tag
- High reproducibility regarding purity and yield for repeated runs on the same column including regeneration between the runs
- Reduced total purification time due to elution of highly concentrated target protein eliminating the need for an extra concentration step
- Convenient prepacked format used in combination with different ÄKTAdesign™ systems

Effective regeneration

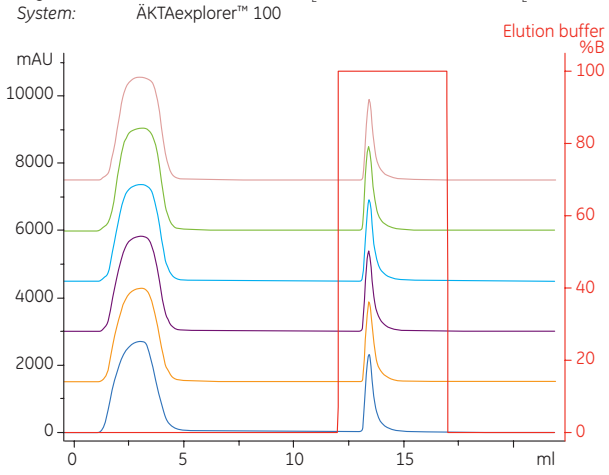
The regeneration of MBPTrap HP is easily performed using sodium hydroxide. Six consecutive purifications of MBP2*-ß-galactosidase in *E. coli* lysate were performed on the same column with regeneration between each run with no reduction in purity or yield (Fig 1). The load was 3.6 mg MBP2*-ß-galactosidase and the final purity was above 95% according to SDS-PAGE (Fig 1 B). The yield was approximately 2.5 mg (70%) for all six runs. This shows the high reproducibility during repeated use of MBPTrap HP in combination with regeneration with 0.5 M NaOH.

Simplified purification of a protein involved in metabolic disease

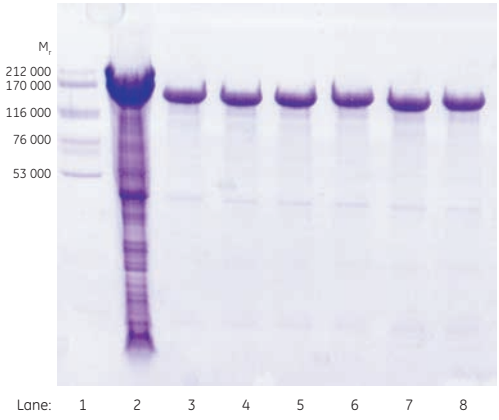
Using the MBPTrap HP column eliminated a concentration step in a purification procedure for medium-chain acyl-CoA dehydrogenase (MCAD). This homotetramer, which is involved in metabolic disease, was purified for stability, folding and kinetic studies. MBPTrap HP replaced the earlier affinity purification step. The highly concentrated target protein was eluted from the MBPTrap HP column in a small volume (Fig 2A). Consequently, the former concentration step prior to final gel filtration could be avoided and significant time saved.

A

Column: MBPTrap HP 1 ml
Sample: MBP2*-ß-galactosidase in *E. coli* lysate, M_r ~158 000
Flow rate: 1 ml/min
(0.5 ml/min for sample application and 0.5 M NaOH)
Binding buffer: 20 mM Tris-HCl, 200 mM NaCl, 1 mM EDTA, 1 mM DTT, pH 7.4
Elution buffer: 10 mM maltose in binding buffer
Regeneration: 3 ml 1.5 M NaCl, 3 ml H_2O, 3 ml 0.5 M NaOH, 3 ml H_2O
System: ÄKTAexplorer™ 100



B

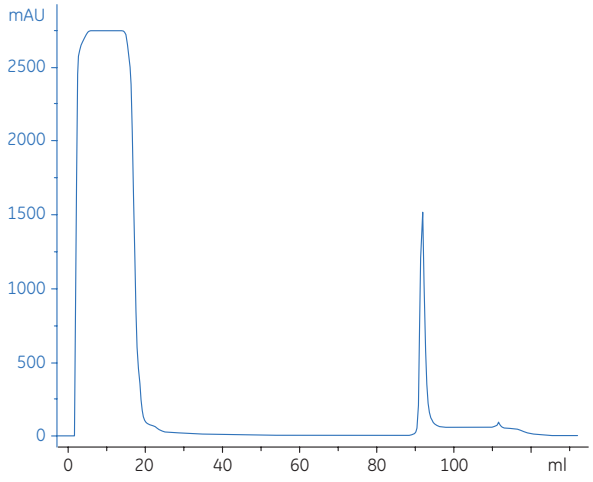


Lane
1: High molecular weight marker, HMW
2: Start material, MBP2*-ß-galactosidase in *E coli* lysate
3: Run 1, eluted pool
4: Run 2, eluted pool
5: Run 3, eluted pool
6: Run 4, eluted pool
7: Run 5, eluted pool
8: Run 6, eluted pool

Fig 1. Repeated purification and regeneration on the same MBPTrap HP column. Absorbance curves at 280 nm (A) and SDS-PAGE analysis (Coomassie™ stained, reduced conditions) (B) of eluted pools from six purification runs.

A

Column: MBPTrap HP 5 ml
Sample: N-terminal MBP-MCAD in *E. coli* lysate, M_r ~85 500
Sample volume: 15 ml
Flow rate: 5 ml/min
(0.5 ml/min during sample loading)
Binding buffer: 20 mM Tris-HCl, 200 mM NaCl, 1 mM EDTA, 1 mM DTT, pH 7.4
Elution buffer: 10 mM maltose in binding buffer
System: ÄKTAprime™



B

Column: Superdex™ 200 pg in XK 16/20
Sample: Eluted fraction from MBPTrap HP 5 ml
Sample volume: 2 ml
Flow rate: 0.4 ml/min
Buffer: 20 mM HEPES, 200 mM NaCl, pH 7.0
System: ÄKTAprime

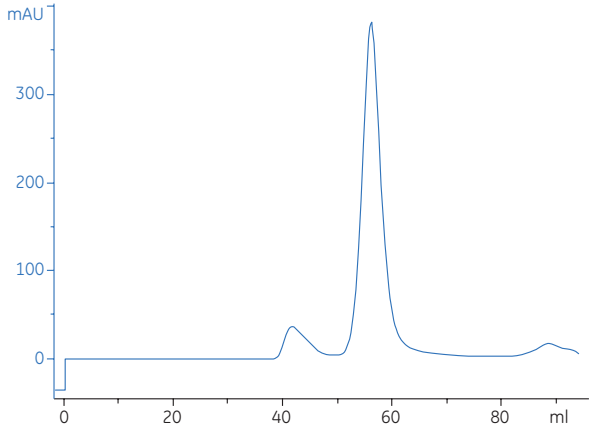
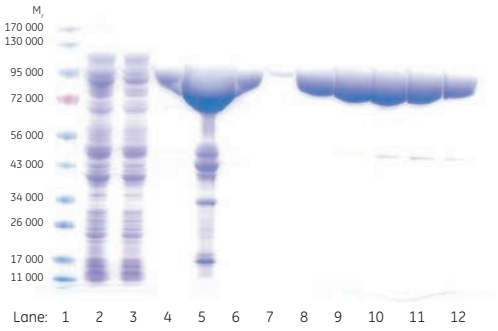


Fig 2. Purification of MBP-MCAD by MBPTrap HP (A) and Superdex 200 pg in XK 16/20 (B). Absorbance curves at 280 nm.



Lane

1: Molecular weight marker
2: Start material, N-terminal MBP-MCAD in *E. coli* lysate, dil. 6×
3: Flow through MBPTrap HP, dil. 6×
4–6: Eluted fractions from MBPTrap HP
7–12: Eluted fractions from gel filtration

Fig 3. SDS-PAGE analysis (Coomassie stained, reduced conditions) of fractions from purification of MBP-MCAD.

The purity of the eluted fractions from MBPTrap HP and gel filtration was determined by SDS-PAGE analysis (Fig 3). As well as the target protein, some additional proteins were detected after the affinity step. This may be due to the presence of truncated variants still having the N-terminal MBP-tag intact, or possibly *E. coli* proteins associated with the target protein (this was not evaluated further). Final purity after gel filtration was high (greater than 95%) according to SDS-PAGE analysis. Final yield was approximately 8.4 mg. As well as cutting total purification time and eliminating the concentration step, the recovery of target protein was also increased due to fewer purification steps.

Automated two-step purification using ÄKTAxpress

A two-step purification procedure was used to purify MBP-tagged Apoptin protein, M_r ~60 000 . The protein was intended for crystallization screening and functional studies. The purification was performed using ÄKTAxpress™ and an affinity (AC) - gel filtration (GF) protocol (Fig 4). Columns used were MBPTrap HP (AC) and HiLoad™ 16/60 Superdex 200 pg (GF). The eluted peak from MBPTrap HP was automatically collected in a loop and injected to the gel filtration column. The position of the fractions from the gel filtration indicated a molecular weight of approximately 120 kDa, while analysis by SDS-PAGE under reduced conditions detected proteins with a molecular weight of 60 kDa (Fig 5). The discrepancy in molecular weight might be due to the presence of dimeric Apoptin or possibly chaperones in the eluted pool. According to literature, the *E. coli* chaperone GroEL, M_r ~60 000, may be associated and co-purified with MBP (Kiser *et al.* Acta Cryst., 2007). By using an antibody against GroEL, the presence of GroEL in the eluted fractions was confirmed by Western blot (data not shown).

AC-column:	MBPTrap HP 5 ml
Sample:	MBP-Apoptin in *E. coli* lysate, M_r ~60 000
Sample volume:	15 ml
Flow rate:	5 ml/min
Binding buffer:	20 mM Tris-HCl, 200 mM NaCl, 1 mM EDTA, 1 mM DTT, pH 7.4
Elution buffer:	10 mM maltose in binding buffer
GF-column:	HiLoad 16/60 Superdex 200 pg
Sample:	Collected pool from MBPTrap HP
Sample volume:	2 ml
Flow rate:	0.3 ml/min
Buffer:	10 mM sodium phosphate, 140 mM NaCl, 0.5 M EDTA, pH 7.2
System:	ÄKTAxpress

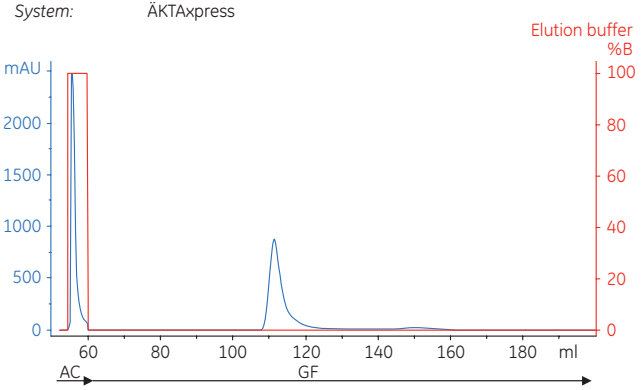
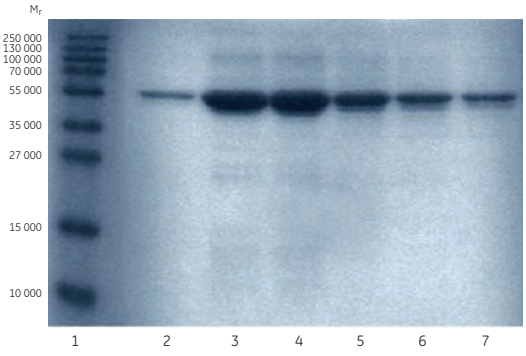


Fig 4. Automated two-step purification of MBP-Apoptin by MBPTrap HP (AC) and HiLoad 16/60 Superdex 200 pg (GF).



Lane

1: Molecular weight marker
2–7: Eluted fractions from gel filtration

Fig 5. SDS-PAGE analysis (Coomassie stained, reduced conditions) of fractions from the gel filtration step.

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