PROTOCOL		
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Title: Protocol– MBP-tag purification- Amylose column		**Page** **Page 1 of 1**

Amylose Column

Materials & Equipment

- Column buffer: 20 mM Tris-HCl pH 7.4 (pH 8 can also be used), 0.2 M NaCl, 1 mM EDTA. **The same buffer is used during cell breaking**.
- Elution buffer: Column buffer + 10 mM maltose (Stock: 0.5 M maltose in 4°C filtered by 0.22 um membrane)
- MQ
- 0.1% SDS
- Storage buffer: Column buffer + EtOH 20%

Note: Unless stated otherwise, all washes are performed at column volume (CV).

Experiment procedure

1. Drain column and wash 3 times with MQ ***(only if column was mounted with EtOH 20%).***
2. Wash column with 5 CV of column buffer.
3. Load your protein onto the column. Collect the FT from column and keep on ice.
4. Wash column with 10 CV of column buffer. Collect the wash.
5. Elution with the elution buffer, collect 5 ml fractions and keep on ice.
6. Measure OD_{280} with quart cuvette. Do not forget to calibrate first with Elution buffer. Stop when OD <0.2.
7. Prepare samples for SDS-PAGE analysis.

Column regeneration

1. Wash 3 column volumes with MQ.
2. Wash 3 column volumes with 0.1% SDS
3. Wash 1 column volume with MQ.
4. Wash 3 times with Store buffer.
5. Keep the column at 4°C. **You can use the same beads only for 5 purifications**.