

WEB312 - SOMT Refolding & HIC

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1 Refolding

- refolded 1.25 ml $1 \frac{mg}{mL}$ SOMT in 25 mL buffer 12 over night at 4°C
- adjusted pH to 7 using 5 N KOH
- took four 2.5 mL aliquot
- added 2.5 mL of each of the following to obtain the half of these concentrations to apply samples A-D to HIC
 - a) 2 M $(NH_4)_2SO_4$
 - b) 1.2 M $(NH_4)_2SO_4$
 - c) 0.6 M $(NH_4)_2SO_4$
 - d) 2 M NaCl
- for SDS PAGE 500 μ l of each sample (5 mL total volume) was precipitated using TCA

ATTENTION: only had about 100 μ l of C for precipitation with TCA and subsequently SDS-PAGE

2 HIC

- prepared four phenyl sepharose columns (1 mL matrix each)
- equilibrated matrix with 3 CV water & 3 CV of each for columns (A-D):
 - a) 1 M $(NH_4)_2SO_4$
 - b) 0.6 M $(NH_4)_2SO_4$
 - c) 0.3 M $(NH_4)_2SO_4$
 - d) 1 M NaCl
- applied 4.5 mL of each sample to column
- washed with 4 mL of solutions (a-d, 1 M, 0.6 & 0.3 M AS and 1 M NaCl, see above)
- eluted with 4 mL of 50 mM KPi pH 7
- for SDS-PAGE 2 mL of each fraction was precipitated using TCA and resuspended in 10 μ l PBS and 2 μ l SDS loading dye (heated to 95 °C and applied to gel)

Table 1: Sample numbering.

No.	Sample	description
1	1 M $(\text{NH}_4)_2\text{SO}_4$	flow through
2		wash
3		elution
A		applied 2 HIC
4	0.6 M $(\text{NH}_4)_2\text{SO}_4$	flow through
5		wash
6		elution
B		applied 2 HIC
7	0.3 M $(\text{NH}_4)_2\text{SO}_4$	flow through
8		wash
9		elution
C		applied 2 HIC
10	1 M NaCl	flow through
11		wash
12		elution
D		applied 2 HIC