WEB317 - SOMT Refolding & ÄKTA HIC

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1 Refolding

- refolded 5 ml 2$\frac{mg}{mL}$ SOMT in 100 mL buffer 12 over night at 4°C
- added 1 Volume (100 mL) of 2 M $(NH_4)_2SO_4$
- adjusted pH to 7 using 5 M KOH → solution turned slightly turbid
- centrifuged to remove precipitate (20.000 x g, 20°C , 30 min)

2 HIC

! **all steps (equilibration, sample injection and elution) were conducted at room temperature !**

- equilibrated 1 mL phenyl sepharose column (HiTrap Phenyl FF (lows sub)) with 5 CV water & 5 CV 1 M $(NH_4)_2SO_4$, 50 mM HEPES pH 7
- applied 50 mL of clarified sample
- eluted stepwise from 1 M $(NH_4)_2SO_4$ to 20 % EtOH (see WEB309), then 70 % EtOH, 0.1 M NaOH and 0.5 M NaOH, and collected 4 mL fractions

3 Activity Test

- using fraction B5 (WEB316), A4 and A6
- control experiment with 20 mM Hepes pH 7

Reaction Mix

0.1 M HEPES pH 7
±0.2 mM Quercetin
0.25 mM SAM
70 μl of corresponding fraction

Table 1: Activity Test setups

sample	~ Experiment/Run/Fraction	Quercetin (uM)	SAM (uM)
A	WEB316/1/B5	200	250
B	WEB316/1/B5	200	0
C	WEB317/1/A4	200	250
D	WEB317/1/A4	200	0
E	WEB317/1/A6	200	250
F	WEB317/1/A6	200	0
G	buffercontrol	200	250

Table 2: Activity test pipetting scheme

sample	Fraction (70 uL)	Quercetin (10 mM)	SAM (5 mM, 73.5%)	Buffer	H_2O
A	WEB316/1/B5	2	6.8	10	11.2
B	WEB316/1/B5	2	0	10	18
C	WEB317/1/A4	2	6.8	10	11.2
D	WEB317/1/A4	2	0	10	18
E	WEB317/1/A6	2	6.8	10	11.2
F	WEB317/1/A6	2	0	10	18
G	buffercontrol	2	6.8	10	11.2

Table 3: Layout Bradford Plate

Well	sample	dilution factor
A3	WEB316 Fr. B12	1
B3	WEB316 Fr. B8	1
C3	WEB316 Fr. B5	1
D3	WEB317 Fr. A4	1
E3	WEB317 Fr. A5	1
F3	WEB317 Fr. A6	1
G3	WEB317 Fr. A7	1
H3	WEB317 Fr. A8	1
A4	WEB317 Fr. A9	1
A5	WEB316 Fr. B12	5
B5	WEB316 Fr. B8	5
C5	WEB316 Fr. B5	5
D5	WEB317 Fr. A4	5
E5	WEB317 Fr. A5	5
F5	WEB317 Fr. A6	5
G5	WEB317 Fr. A7	5
H5	WEB317 Fr. A8	5
A6	WEB317 Fr. A9	5