WEB319 - SOMT Refolding & ÄKTA HIC

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1 Refolding

- refolded 2.5 ml 1$\frac{mg}{mL}$ SOMT in 50 mL reducing buffer 12 (0.1 M Mes, 10% glycerol, 0.5 M Arginine*HCl, 2 mM $MgCl_2$, 2 mM $CaCl_2$, 0.5 mM Tween-80, 10 mM NaCl, 0.5 mM KCl, 5 mM DTT pH 5.5) over night at 4°C
- added 1 Volume (50 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 A4, A6 and A9
- control experiment with 20 mM Hepes pH 7
- 6 x MM for each substrate group
  → **Group 1:** Naringenin, Daidzein, ED
  — **Group 2:** Genistein, Quercetin, HED
  — **Group 3:** Apigenin, Hesperetin

Reaction Mix

0.1 M HEPES pH 7
0.2 mM substrate
0.25 mM SAM
in eluate

Mastermix Group 1 (5x)

50 μl 1 M HEPES pH 7
10 μl 10 mM Naringenin, Daidzein, ED
34 μl 5 mM SAM
36 μl H_2O

Mastermix Group 2 (5x)

50 μl 1 M HEPES pH 7
10 μl 10 mM Genistein, Quercetin, HED
34 μl 5 mM SAM
36 μl H_2O

Mastermix Group 3 (6x)

50 μl 1 M HEPES pH 7
10 μl 10 mM Apigenin, Hesperetin
34 μl 5 mM SAM
46 μl H_2O

Reaction:

30 μl mastermix
70 μl eluate fraction

Reaction for WEB316/B5:

2 μl Quercetin in DMSO or MeOH
10 μl 1 M Hepes pH 7
18 μl ddH_2O
70 μl eluate fraction

Reaction Conditions: incubate 2 h at 30 °C

sample	Fraction	Substrate group
A	A4	1
B	A4	2
C	A4	3
D	A6	1
E	A6	2
F	A6	3
G	A9	1
H	A9	2
I	A9	3
J	buffer	1
K	buffer	2
L	buffer	3
O	WEB316/B5	Quercetin from MeOH stock
P	WEB316/B5	Quercetin from DMSO stock