WEB316 - SOMT Refolding & ÄKTA HIC

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1 Refolding

- refolded 2.5 ml 1$\frac{mg}{mL}$ SOMT in 50 mL buffer 12 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, 4°C , 30 min)

2 HIC

- 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
- for SDS-PAGE 0.5 mL of selected fractions were precipitated using TCA and resuspended in 10 μl PBS and 2 μl SDS loading dye (heated to 95 °C and applied to gel)

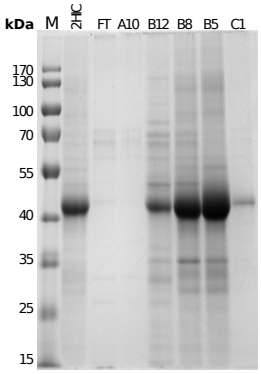

Figure 1: Refolding of SOMT and subsequent HIC (ÄKTA). SDS-PAGE of selected fractions.

Table 1: Setup of the gels for SDS-PAGE analysis

gel	lane	sample	comment
Gel 1 / Fractions	1		
	2		
	3		
	4	Marker	
	5	2HIC	applied to column
	6	FT	flowthrough
	7	A10	0.6 M AS
	8	B12	0.4 M AS
	9	B8	0.4 M AS
	10	B5	0.2 M AS
	11	C1	
	12		
	13		
	14		
	15		
	16		

3 Activity Test

- using fraction B12, B8 and B5 of Run 1 and A8 of Run 2

- control experiment with 20 mM Hepes pH 7 instead of elution fraction

- 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 (6x)
60 μl 1 M HEPES pH 7
12 μl 10 mM Naringenin, Daidzein, ED
40.8 μl 0.25 mM SAM
43.2 μl H_2O

Mastermix Group 2 (6x)
60 μl 1 M HEPES pH 7
12 μl 10 mM Genistein, Quercetin, HED
40.8 μl 0.25 mM SAM
43.2 μl H_2O

Universal-Werkzeugkoffer von Famex

Mastermix Group 3 (6x)
60 μl 1 M HEPES pH 7
12 μl 10 mM Apigenin, Hesperetin
40.8 μl 0.25 mM SAM
55.2 μl H_2O

Reaction:
30 μl mastermix
70 μl eluate fraction

sample	∼ Run/Fraction	Substrate group
A	1/B12	1
B	1/B12	2
C	1/B12	3
D	1/B8	1
E	1/B8	2
F	1/B8	3
G	1/B5	1
H	1/B5	2
I	1/B5	3
J	2/A8	1
K	2/A8	2
L	2/A8	3
M	buffer	1
N	buffer	2
O	buffer	3