

WEB315 - SOMT2 expression from pET20b in *E.coli* C43(DE3)

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1 Expression

- 3 mL pre-culture of *E.coli* C43(DE3) pET20b SOMT2 from glycerol stock in LB-medium with 200 $\frac{\mu g}{mL}$ ampicillin
- incubation at 37°C and 220 rpm over night
- next morning → measured OD⁶⁰⁰
- inoculated 100 mL culture in TB medium to an OD of ~0.06 (1.7 mL)
- incubated at 37°C/220rpm for 1.5 h
- incubated at 30°C for 1 hour
- induced with 1 mM IPTG and incubated at 30°C for 5 hours
- collected cells by centrifugation at 4°C and 10.000 x g for 10 min
- drained and weighed cell pellet

time	OD ⁶⁰⁰	comment	SDS-PAGE sample
0935	3.578	inoculated main culture with 1.7 mL	
1113	0.307	set at 30°C	
1217	0.72	induce with 1 mM IPTG	X (VI, 1 ml)
1700	5.341	1.22 g cell pellet	X (NI, 900μl)

2 Periplasmic Subfractionation

- resuspended pellet in 100 mL (80 mL per gram wet weight) buffer A
- stirred suspension slowly at room temp for 10 min
- centrifuged at 10.000 x g, 4°C, 10 min
- drained pellet

- resuspended pellet in 100 mL ice cold 5 mM MgSO_4
- stirred suspension slowly on ice for 10 min
- centrifuged at 10.000 x g, 4°C, 10 min
- collected supernatant (100 mL) and adjusted to 1 M $(\text{NH}_4)_2\text{SO}_4$ using 100 mL 2 M $(\text{NH}_4)_2\text{SO}_4$
- adjusted pH to 7 using 3.1 mL K_2HPO_4 and 1.9 mL KH_2PO_4

3 Phenyl Sepharose HIC Column

- 1 mL phenyl sepharose matrix
- washed with water and equilibrated with 50 mM KPi, 1 M $(\text{NH}_4)_2\text{SO}_4$ pH 7
- applied sample (200 mL) to column at room temp
- washed/eluted stepwise with (3 mL each):
 - 1) 1 M $(\text{NH}_4)_2\text{SO}_4$, 50 mM KPi pH 7
 - 2) 0.6 M $(\text{NH}_4)_2\text{SO}_4$, 50 mM KPi pH 7
 - 3) 0.3 M $(\text{NH}_4)_2\text{SO}_4$, 50 mM KPi pH 7
 - 4) 50 mM KPi pH 7
 - 5) 20 % EtOH
 - 6) **5 mL 0.1 N NaOH**
- collected 3 mL fractions
- for SDS-PAGE 1 mL of each fraction was precipitated with TCA

sample	comment	SDS-PAGE sample	resusp. in PBS [μl]
1	Periplasma Prep	PP	10
2	PP after AS addition	AS	10
3	applied to HIC	2HIC	10
4	HIC flowthrough	FT	10
5	HIC Wash	1 M	100
6	HIC Elution 0.6 M	0.6	100
7	HIC Elution 0.3 M	0.3	100
8	HIC Elution 0 M	0	100
9	HIC Elution 20% EtOH	EtOH	100

3.1 Observations

- after about 50 mL of sample applied to HIC flow slowed down notably (maybe aggregated protein, DNA etc. on column)
- washing with 0.1 N NaOH speeds up flow again

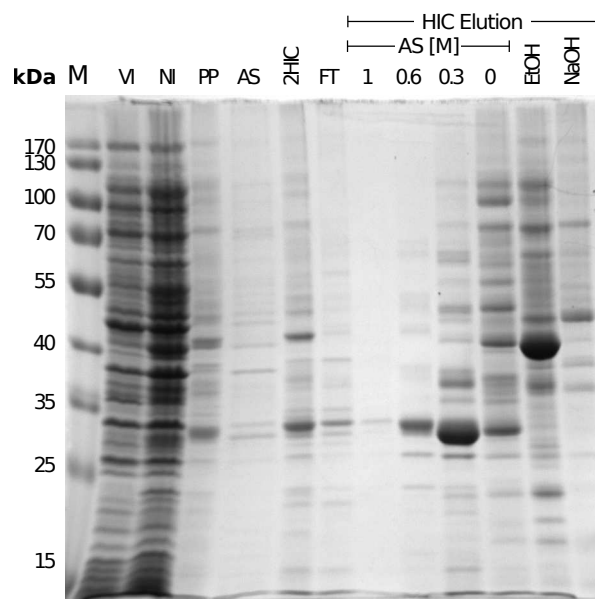


Figure 1: SDS-PAGE of Expression and fractions from HIC column. **VI** - pre-induction, **NI** - post-induction, **PP** - periplasm fraction, **AS** - pellet after addition of 1 M $(\text{NH}_4)_2\text{SO}_4$ to the periplasm fraction, **2HIC** - supernatant (applied to HIC), **FT** - flow through, **1** through **0** correspond to the AS concentrations during the elution, **EtOH** - washed with 20% EtOH, **NaOH** - washed with 0.1 N NaOH

4 Activity Test

- using the 20% ethanol fraction
- control experiment with 20% EtOH instead of elution fraction

Reaction Mix

0.1 M HEPES pH 7
0.2 mM Naringenin
0.2 mM Daidzein
0.2 mM Eriodictyol
0.25 mM SAM
in eluate

Reaction:

10 μl 1 M HEPES pH 7
2 μl 10 mM Naringenin
2 μl 10 mM Daidzein
2 μl 10 mM Eriodictyol
6.8 μl 5 mM SAM (73.5%)
77.2 μl eluate (EtOH fraction)

sample	substrates	description
1+2	Naringenin, Daidzein, ED	
3+4	Quercetin, Genistein	
0/1+2	Naringenin, Daidzein, ED	control
0/3+4	Quercetin, Genistein	control

5 Activity Test - Day 2

- using the 20% ethanol fraction
- control experiment with 20% EtOH instead of elution fraction

Reaction Mix

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

Reaction:

10 μ l 1 M HEPES pH 7
 2 μ l 10 mM Quercetin 6.8 μ l 5 mM SAM
 (73.5%)
 77.2 μ l eluate (EtOH fraction)

sample	~ Enzym (mg/mL)	SAM (uM)	Quercetin (uM)	EtOH (%)
A	0.2	200	200	20
B	0.1	200	200	10
C	0.02	200	200	2
D	0.2	1000	200	20
E	0.2	200	0	20
F	0	200	200	20
G	0.2	0	200	20

sample	Fraction	SAM	Quercetin (10 mM)	1 M Hepes pH 7	H ₂ O
A	81.2	6.8	2	10	-
B	40.6	6.8	2	10	40.6
C	8.12	6.8	2	10	73.08
D	40.6	27.2	2	10	20.2
E	81.2	6.8	0	10	2
F	-	6.8	2	10	81.2
G	81.2	0	2	10	6.8