

# WEB323 - 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 OD<sup>600</sup>
- 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 <sup>600</sup>	comment	SDS-PAGE sample
1100	6.0	inoculated main culture with 1 mL	
1230	0.44	set at 30°C	
1330	0.846	induce with 1 mM IPTG	X (VI, 1 ml)
1830	5.55	~1 g cell pellet	forgot to take sample

## 2 Periplasmic Subfractionation

- resuspended pellet in 100 mL (80 mL per gram wet weight) buffer A (30 mM Tris/HCl, 20% (w/v) D-Saccharose, 1 mM EDTA pH 8)
- 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  $\text{MgSO}_4$
- 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  $\text{K}_2\text{HPO}_4$  and 1.9 mL  $\text{KH}_2\text{PO}_4$

### 3 Phenyl Sepharose HIC Column (Fig. 1a)

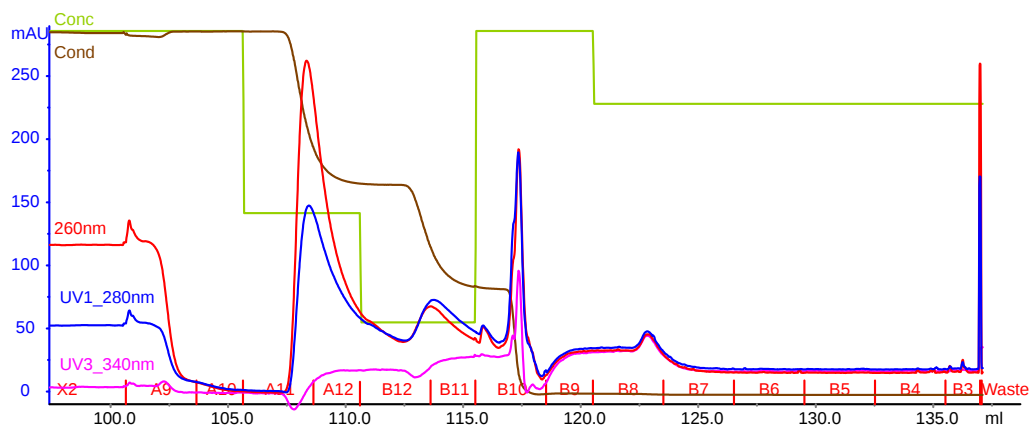
- HiTrap phenyl sepharose FF low-sub 1 mL
- washed with water and equilibrated with 50 mM Hepes/NaOH, 1 M  $(\text{NH}_4)_2\text{SO}_4$  pH 7
- applied sample (100 mL) to column at room temp
- washed/eluted stepwise with (5 mL each):
  - 1) 1 M  $(\text{NH}_4)_2\text{SO}_4$ , 50 mM Hepes/NaOH pH 7
  - 2) 0.5 M  $(\text{NH}_4)_2\text{SO}_4$ , 50 mM KPi pH 7
  - 3) 0.2 M  $(\text{NH}_4)_2\text{SO}_4$ , 50 mM KPi pH 7
  - 4) 50 mM Hepes/NaOH pH 7
  - 5) 20 % EtOH, 50 mM Hepes/NaOH pH 7
- collected 3 mL fractions
- for SDS-PAGE 1 mL of each fraction was precipitated with TCA

#### 3.1 Buffers:

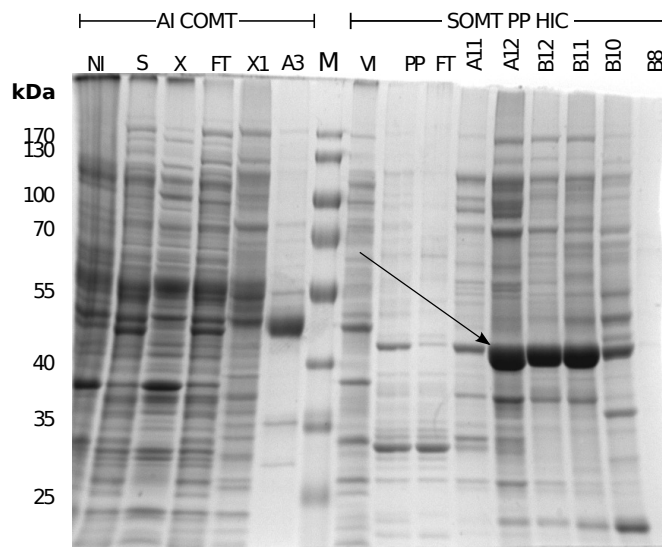
- **inlet A1** – Buffer A.1: 50 mM Hepes, pH 7.0
- **inlet A2** – Buffer A.2: 96% EtOH
- **inlet B1** – Buffer B.1: 1 M ammonium sulfate, 50 mM Hepes, pH 7.0
- **inlet B2** – Buffer B.2: 50 mM Hepes, pH 7.0

### 4 Activity Test

- using fraction A12, B12 and B11
- control experiment with 20 mM Hepes pH 7
- 4 x MM for each substrate group
  - **Group 1:** Naringenin, Daidzein, Quercetin



(a) HIC Run of periplasma preparation. Gradient (green line): 100% Buffer B (10 CV), 50% B (5 CV), 20% B (5 CV) – change inlets to A2 (96% EtOH) and B2 (50 mM Hepes pH 7) – 100% B (5 CV), 80% B (15 CV).



(b) SDS-PAGE of purification (only to the right of the marker). The arrow indicates the SOMT protein.

Figure 1: Hydrophobic interaction chromatography (HIC) using HiTrap Phenylsepharose 1 mL (lowsub) and SDS-Gel of collected fractions.

**Reaction Mix**

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

**Mastermix Group 1 (4x)**

40  $\mu$ l 1 M HEPES pH 7  
 8  $\mu$ l 10 mM Naringenin, Daidzein, Quercetin  
 27.2  $\mu$ l 5 mM SAM  
 28.8  $\mu$ l H<sub>2</sub>O

**Mastermix Group 2 (4x)**

40  $\mu$ l 1 M HEPES pH 7  
 8  $\mu$ l 10 mM Naringenin, Daidzein, Quercetin  
 0.8  $\mu$ l 1M DTT  
 27.2  $\mu$ l 5 mM SAM  
 28  $\mu$ l H<sub>2</sub>O

**Reaction:**

30  $\mu$ l mastermix  
 70  $\mu$ l eluate fraction

**Reaction Conditions:** incubate 2 h at 30 °C

sample	Fraction	Reducing	comment
A	A12	no	decoloration
B	B12	no	decoloration
C	B11	no	decoloration
D	A12	yes	decoloration
E	B12	yes	decoloration
F	B11	yes	decoloration
G	buffer	no	visibly more yellow than reactions with enzyme
H	buffer	yes	visibly more yellow than reactions with enzyme