

# Western Blotting for SAA Secretion

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## Sample collection

1. Prepare overnight broth cultures of *E. coli* cells transformed with the SAA expression construct and pSB1AC3 plasmid backbone (control cells)
2. Re-suspend 500ul of each culture in 50ml LB media in a 125mL bottle. Incubate the bottles on 37°C shaker.
3. Collect samples at 0hr time-point by transferring 3ml of the each 50ml culture into a 10ml falcon tube.
4. Pellet cells by centrifugation (4000rpm, 15 minutes) at room temperature.
5. Transfer the supernatant into a new 10ml falcon tube. Store cell pellets and supernatants at -20°C.
6. Repeat step 3 to 5 to collect samples for 2hr, 4hr, and 12hr time-point.

## Sample preparations for SDS-PAGE

7. Transfer 55ul of the supernatant samples into a microcentrifuge tube. Add 11ul of 6X SDS sample buffer into each tube. Boil samples at 100°C for 5 minutes.
8. Prepare 10X dilutions of the supernatant samples by mixing 5.5ul of original supernatants, 49.5ul of ddH<sub>2</sub>O, and 11ul of SDS sample buffer in microcentrifuge tubes.
9. Resuspend the cell pellets in 2ml of ddH<sub>2</sub>O.
10. Repeat step 7 and 8 to prepare cell lysate samples.
11. Repeat step 7 and 8 to prepare positive control samples - HDL derived from inflamed mice (obtained from Dr. Deeley's lab at Queen's Cancer Research Institute)

## Western blotting

Gel recipe:

7.5% gel (for 1mm spacer)		
reagents	separation gel (for 4)	stacking gel (for 4)
H <sub>2</sub> O	20ml	6ml
Buffer	10ml	2.5ml
Acryl (30:08)	10ml	1.5ml
10% APS	400ul	100ul
TEMED	40ul	10ul

12% gel (for 1mm spacer)		
reagents	separation gel (for 4)	stacking gel (for 4)
H <sub>2</sub> O	7ml	6ml
Buffer	5ml	2.5ml
Acryl (30:08)	8ml	1.5ml
10% APS	200ul	100ul
TEMED	20ul	10ul

Gel was run at 20-30mA per gel, and the voltage should be lower than 150V. Wait until the dye front has ran off the gel.

12. cut 2 pieces of whatman filter paper to 5.5X8.5cm
13. cut a piece of membrane to 5.5X8.5cm
14. soak the membrane in MeOH
15. lay the membrane on top of the transfer buffer
16. when the gel is finished running, take the apparatus apart.
17. remove the stacking gel and wash the separation gel in transfer buffer for 10min.
18. wet the transfer apparatus with transfer buffer.
19. wet the filter paper and place one on the apparatus.
20. place the membrane on the filter paper.
21. place the gel on the membrane.
22. place the last filter on top
23. wet with more transfer buffer
24. close the apparatus
25. run 40min at 100mA/gel, but do not let the voltage >25V.
26. rinse membrane in TBST
27. Block in 5% skim milk for 1hr.
28. Incubate nitrocellulose blots with 1° antibody (whole SAA) at 4°C overnight.
29. Wash blots 3X each 15minutes in TBST.
30. Incubate blots with 2° anti-rabbit antibody for 1hr at room temperature.
31. add luminescence kit and agitate for 30s/side
32. expose and develop x-ray film.
33. to strip: 0.2N NaOH for 10min at room temp.