

Protein Purification

Setup of Purification:



2018-09-28

- We purified ALDH2*1 and ALDH2*2 by following the same protocol from before to run functional tests under realistic conditions
 - The results can be found in our Functional Test Notebook.
 - Our results showed that our purified enzymes are able to significantly metabolize NAD⁺, even better than that at 25 degrees.

2018-9-15

- We purified ALDH2*1 and ALDH2*2 by following the same protocol from before

*The protein concentration of the ALDH2*1 eluate was found to be 0.1 mg/mL and ALDH2*2 to be 0.2 mg/mL

- We ran 3 sets of functional tests at room temperature in water, the results can be seen in from our functional test excel sheet.
 - We showed that our purified proteins is able to efficiently reduce NAD⁺. In addition, ALDH2*1 was more efficient at reducing NAD⁺ than ALDH2*2

2018-9-14

- Grow 200 ml cultures (LC) of HIS-tagged ALDH2*1 and ALDH2*2 E. coli

2018-9-06

- We ran a functional test in 37 C with the purified protein from last week.
 - We used ALDH2*1, ALDH2*2, and just elution buffer as negative control

	0	5	10	15	20	25	30	35	40
BOB	0.191	0.2	0.205	0.206	0.179	0.181	0.183	0.185	0.188
MUT	0.174	0.203	0.212	0.209	0.213	0.214	0.216	0.218	0.22
ELUTION BUF	0.205	0.216	0.219	0.22	0.223	0.225	0.227	0.228	0.229
time	5:58	6:03	6:08	6:13	6:18	6:23	6:28	6:33	6:38

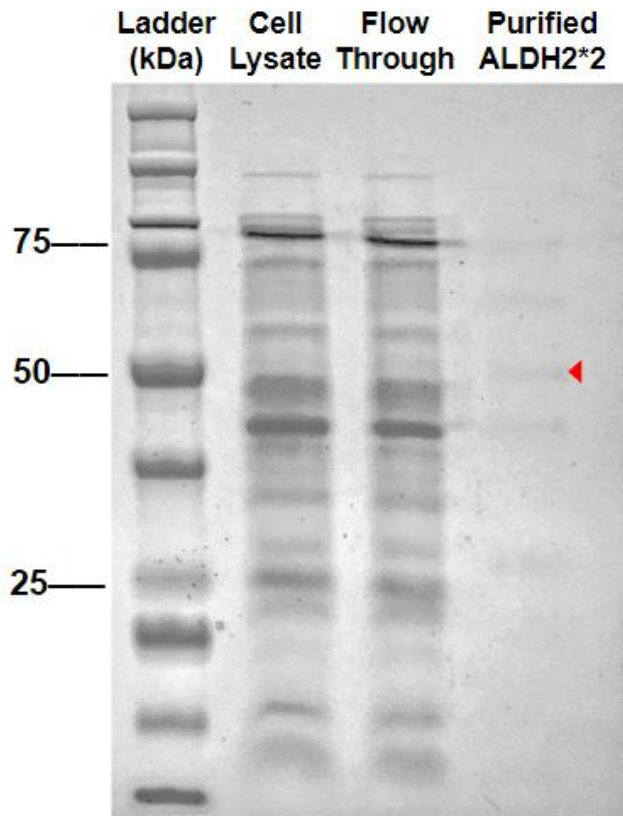
- However, the results were inconclusive. We suspect that this is due to the degradation of proteins. Therefore, we plan to run functional test immediately after purification next time.

2018-9-02

- We purified ALDH2*1 and ALDH2*2 by following the same protocol from last week that worked

*The protein concentration of the ALDH2*1 eluate was found again to be 0.1 mg/mL and ALDH2*2 to be 0.2 mg/mL

- We ran a SDS page with the flow through of the ALDH2*2 purification, raw ALDH2*2 cell extract, and purified ALDH2*2 eluate



- We see a band just around 50 kDa, showing that ALDH2*1 is present. ALDH2*1 is 56 kDa

2018-9-01

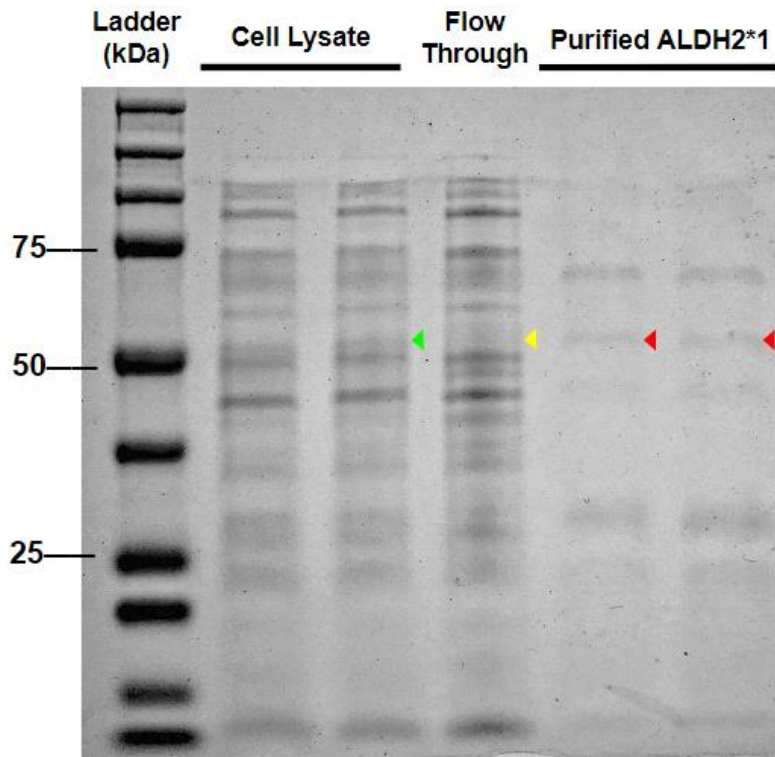
1. Grow 200 ml cultures (LC) of HIS-tagged ALDH2*1 and ALDH2*2 E. coli

2018-8-25

- Purified ALDH2*1 by following the same protocol as before, but we poured in elution buffer twice to attain more purified ALDH2*1 from the column

*The protein concentration of the eluate was found to 0.1 mg/mL

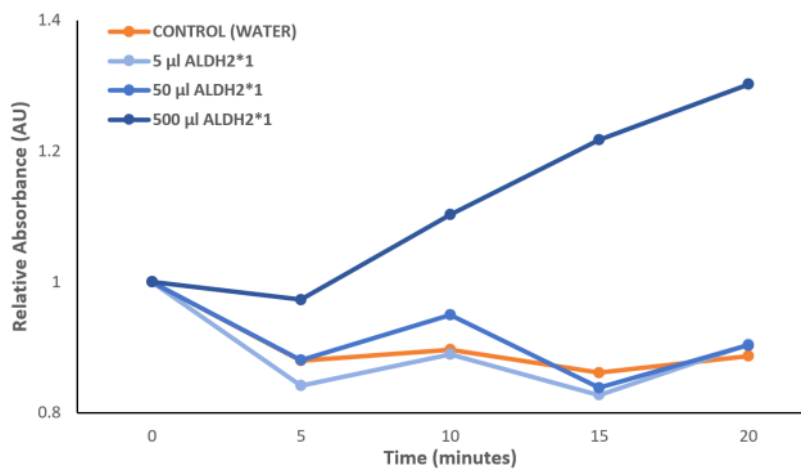
- We ran a SDS page with raw cell extract, flow through, and eluate to make sure that ALDH2*1 is purified:



○ We do see a band just above 50 kDa, showing that ALDH2*1 is present. ALDH2*1 is 56 kDa

- We ran a prelim functional test to determine the optimal purified enzyme amount to use in functional test, either 5 uL, 50 uL, or 500 uL. Our results show that, just as expected, 500 uL seem to be the best amount of purified proteins to testing ALDH2*1 activity

	0	5	10	15	20
500	0.133	0.146	0.142	0.2	0.218
50	0.127	0.126	0.125	0.125	0.119
5	0.18	0.178	0.168	0.171	0.17
water	0.12	0.122	0.117	0.12	0.126



2018-8-24

1. Grow 200 ml cultures (LC) of HIS-tagged ALDH2*1 E. coli

2018-8-18

- Purified ALDH2*1 by following the following protocol

Part I: Cell Extract Preparation

1. Centrifuge sample at 5,000 g, 10 mins., and 4 degrees Celsius to harvest cells
2. Completely resuspend pellet in 20 mL xTractor lysis buffer
3. Incubate 10 minutes at room temperature with gentle shaking
4. Centrifuge sample at 13,300 rpm, 20 mins, 4 degrees Celsius to spin down insoluble debris
5. Transfer supernatant to a new tube
6. Filter cell extract through a 0.45 um syringe filter

Part II: His-tagged protein purification:

1. Remove pre-packed, pre-charged His GraviTrap column cap (GE Health Cat. No. 11-0033-99) and pour out buffer as clean as possible
2. Add 10 ml of washing buffer, cut open the tip of the column to start the flow
3. Allow washing buffer to flow through
4. Load cell extract and let it flow through
5. Add 10 mL washing buffer and let it flow through
6. Add 3.0 mL eluting buffer and let it flow through
7. Assay purified protein

The protein concentration of the eluate was found to be zero :(

2018-8-17

1. Grow 200 ml cultures (LC) of HIS-tagged ALDH2*1 E. coli