ASAP WNV mutant live Purification

PAGE22-02017

Author: **Kot, Ekaterina**
Date Started: **2022-Nov-28**
Experiment Started:
Projects: **Purification**
Related Pages:
Referenced by:
Tags: **ASAP**

Purification Protocol

Base Buffer: 10 mM HEPES pH7.5, 500 mM NaCl, 5 % glycerol, 0.5 mM TCEP

Dissolved pellet (114.2g) in 500mL Base Buffer (+5mM imidazole).
Sonicate (5sec on/5sec off for 15 min)
Centrifuge 30,000g 1hr 12C
Add supernatant to 12mL of TALON resin
Incubate while spinning for 20min at 4C
Centrifuge 1,000g 10min 12C
Pack into a column
Wash 1: 500mL of Base Buffer with 5mM imidazole
Wash 2: 250mL of Base Buffer with 15mM imidazole
Elution: 30mL of Base Buffer with 150mM imidazole

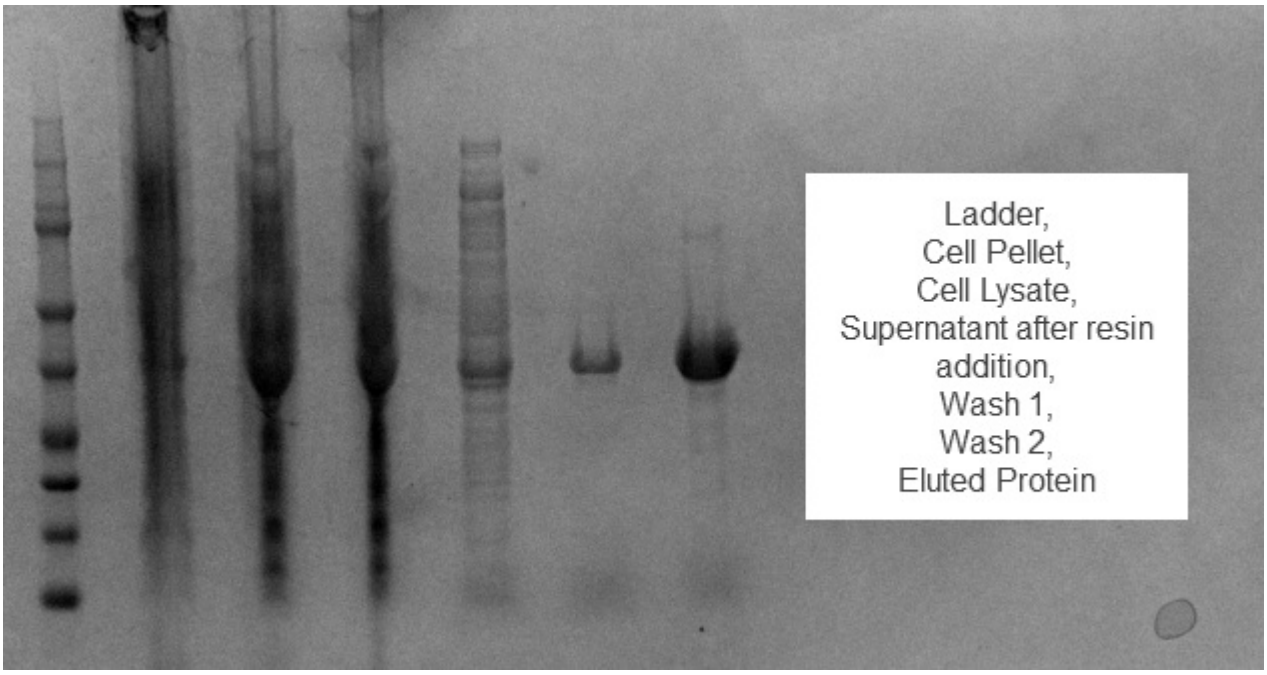
Concentration: 1.474 mg/mL (30mL) [44mg yield]

Protein flash frozen and stored at -80C

Dialyze 30mL sample o/n against 4L of Base Buffer after adding 1:10 TEVsh

---

SDS-PAGE after IMAC



Observations

Amount of resin calculated based on 15 mg/mL binding capacity of TALON, but next time use 10 mg/mL estimate instead, as might be losing protein.

Protein present in Wash 2 - next time use lower imidazole concentration.

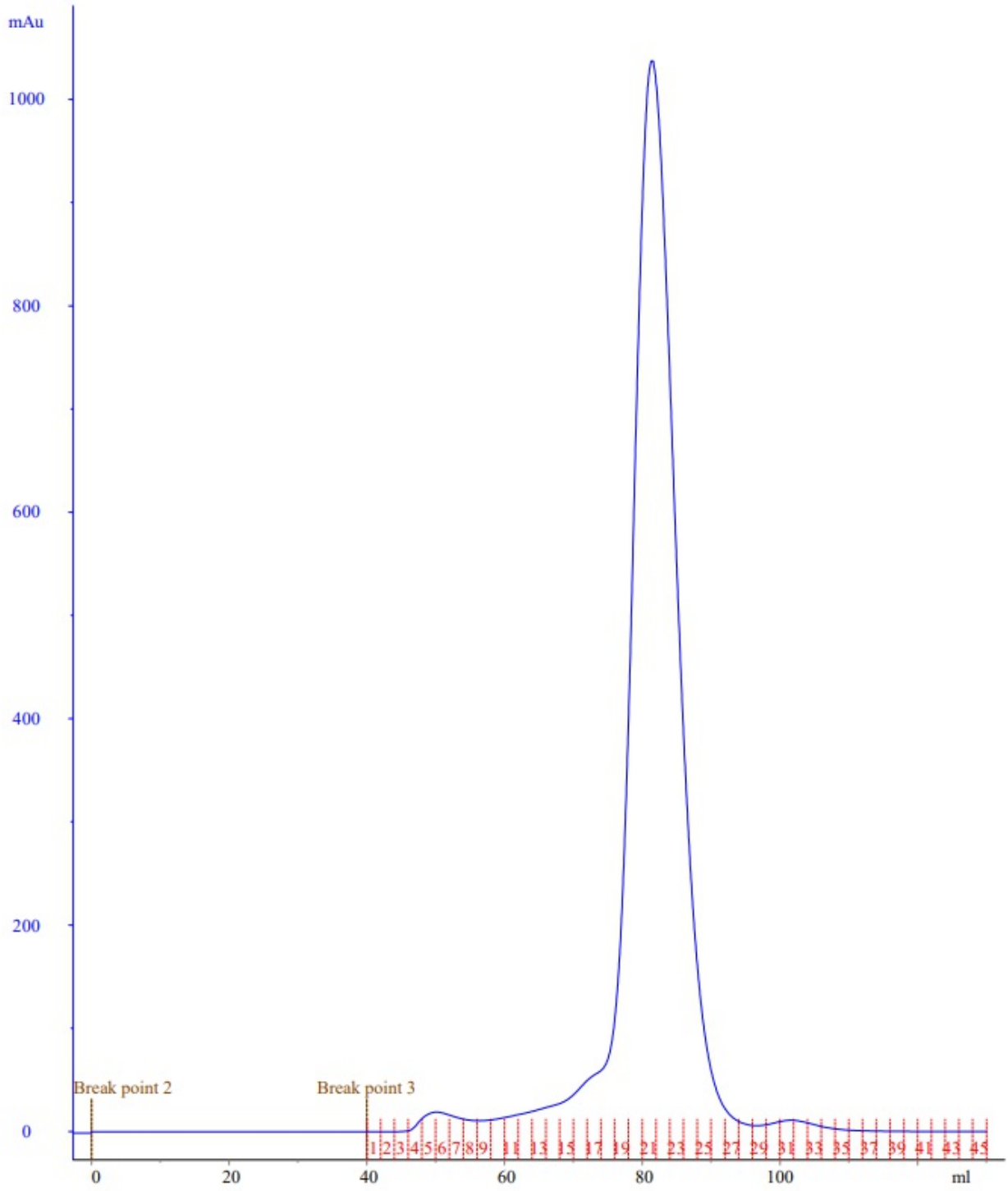
Reverse IMAC and SEC

Reapply sample to the 12mL TALON column adding 12mL of Base Buffer afterwards
Wash column with 20mL Base Buffer + 20mM imidazole

Concentrated to around 5 mL and loaded onto 125 mL Superose 12 PG column ran at 1.5 ml/min at RT using Base Buffer as mobile phase

After SEC concentration was 2.078 mg/mL in 8mL [16.6mg yield]

SEC trace

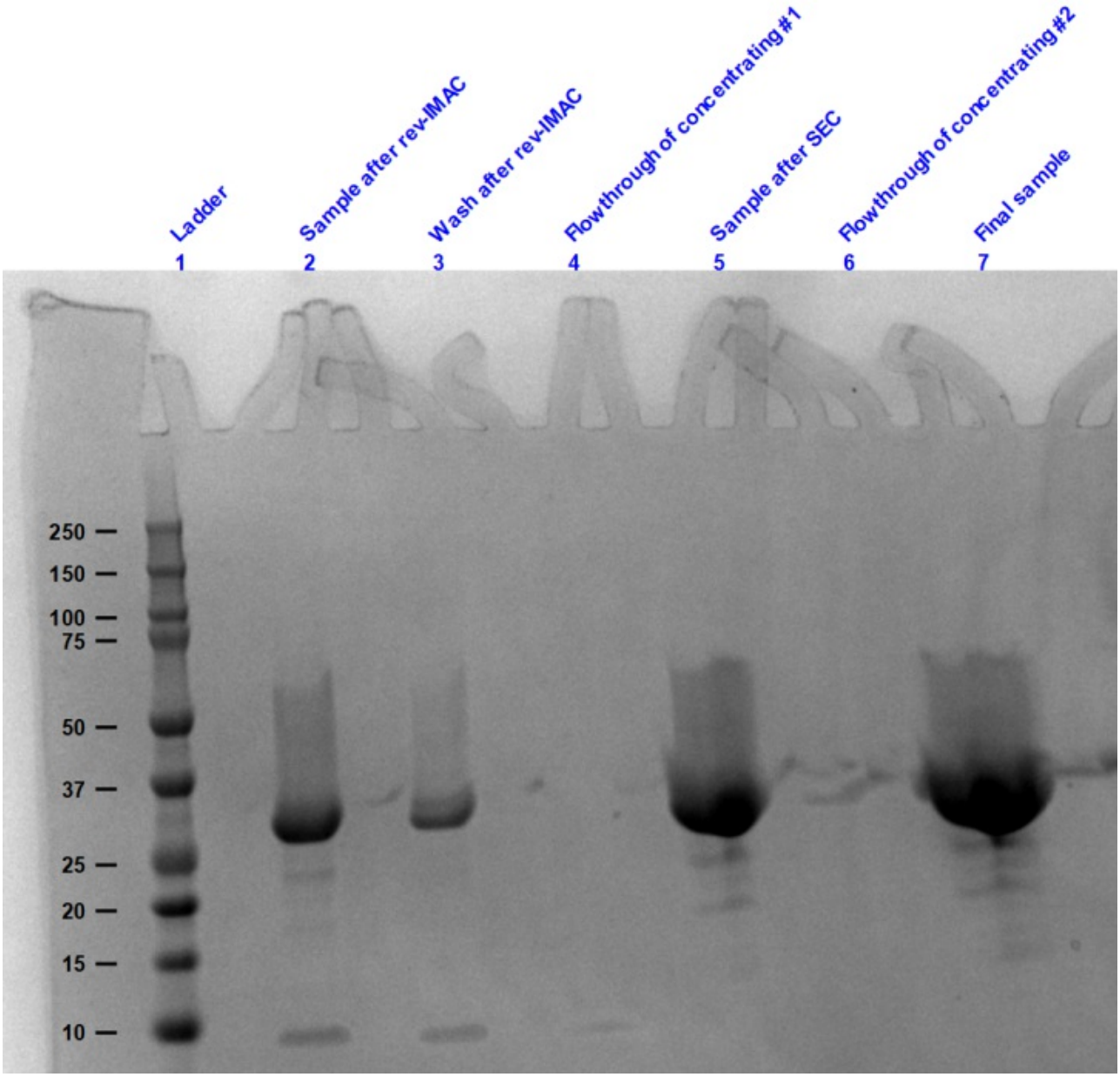


Final Yield

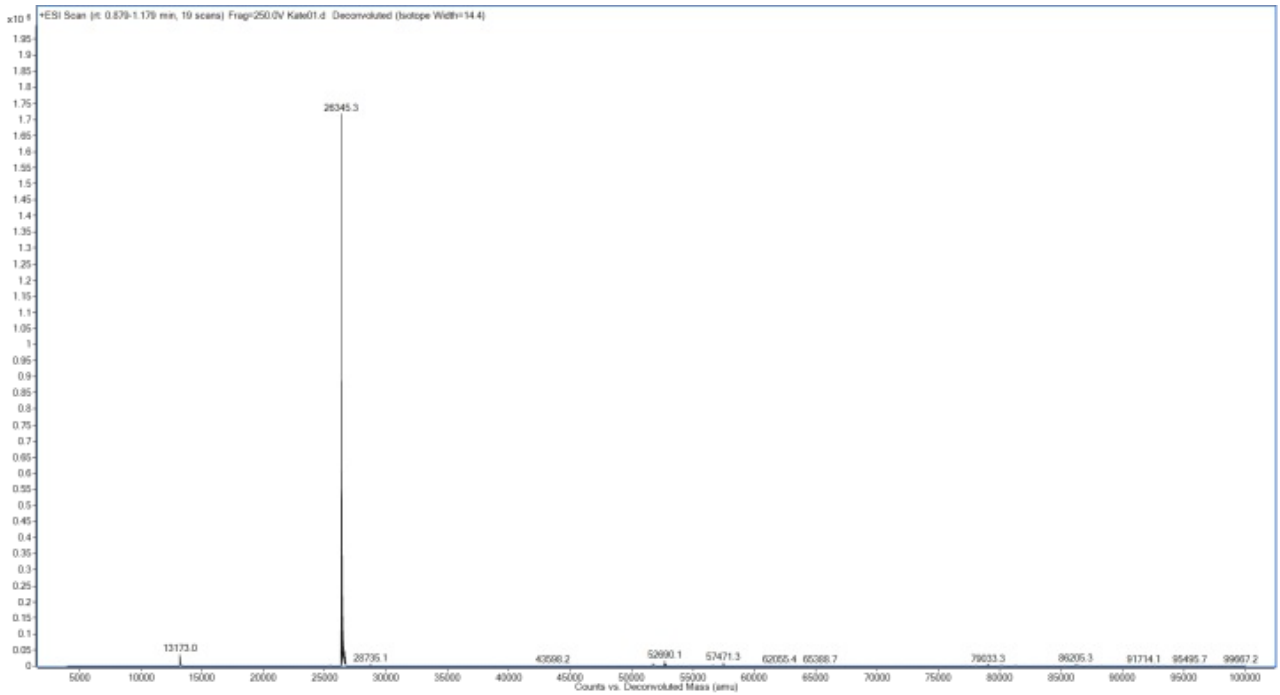
Final concentration: 38.1 mg/mL
Flash-frozen in 2 x 100uL aliquots

Final yield: 7.6 mg

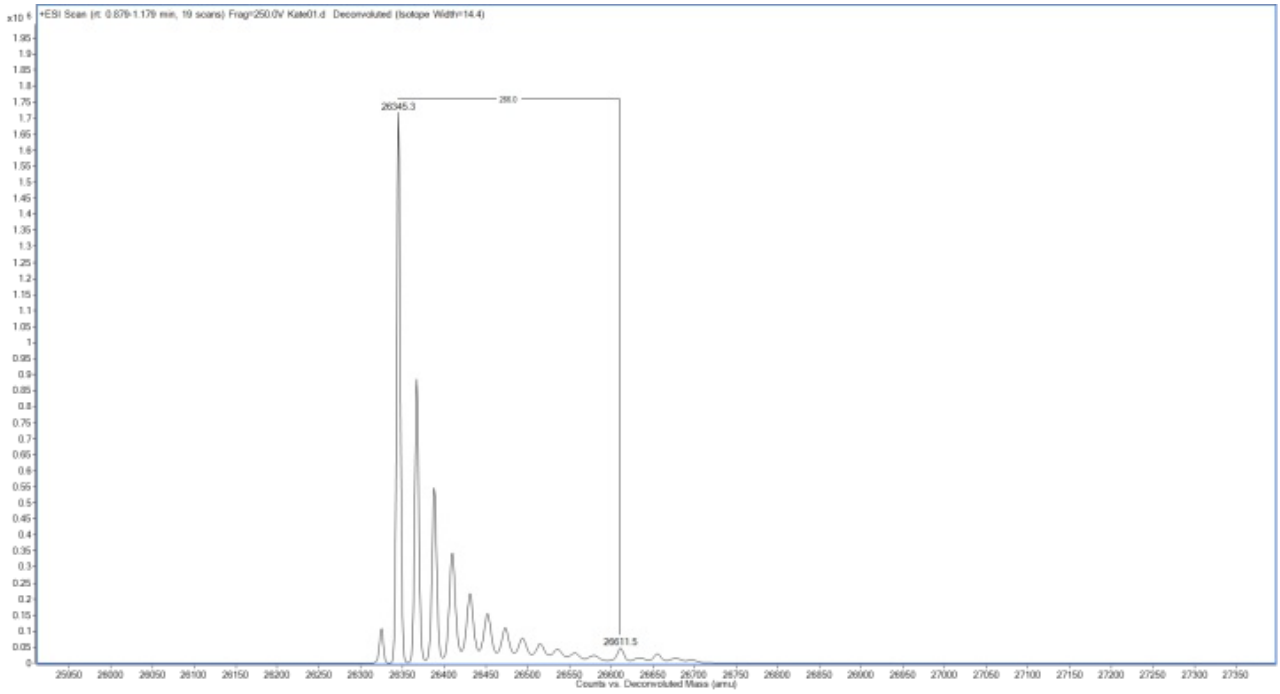
SDS-PAGE after rev-IMAC


Ladder
1
Sample after rev-IMAC
2
Wash after rev-IMAC
3
Flowthrough of concentrating #1
4
Sample after SEC
5
Flowthrough of concentrating #2
6
Final sample
7
250
150
100
75
50
37
25
20
15
10

MS Result



MS Result



Mass Comparison

MS Experimental Mass - 26345.3 Da
Theoretical Mass Expected - 26344.6 Da

Match