SEPTEMBER

WEEK 1

SDS PAGE of Purified Protein

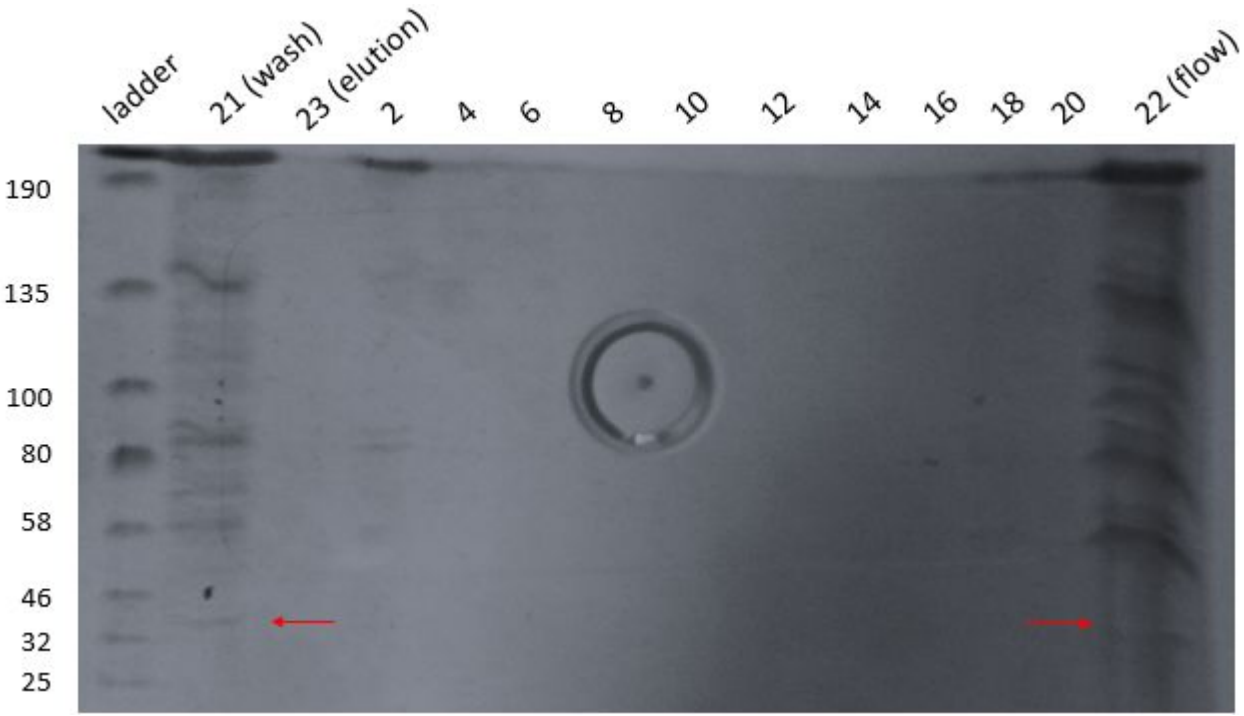
01/09/21

- The collected fractions were run on SDS along with collected wash through and flow through.
- Stacking Gel : 5 %
- Resolving gel : 10 %

SDS PAGE - Gel 1

Annotations:

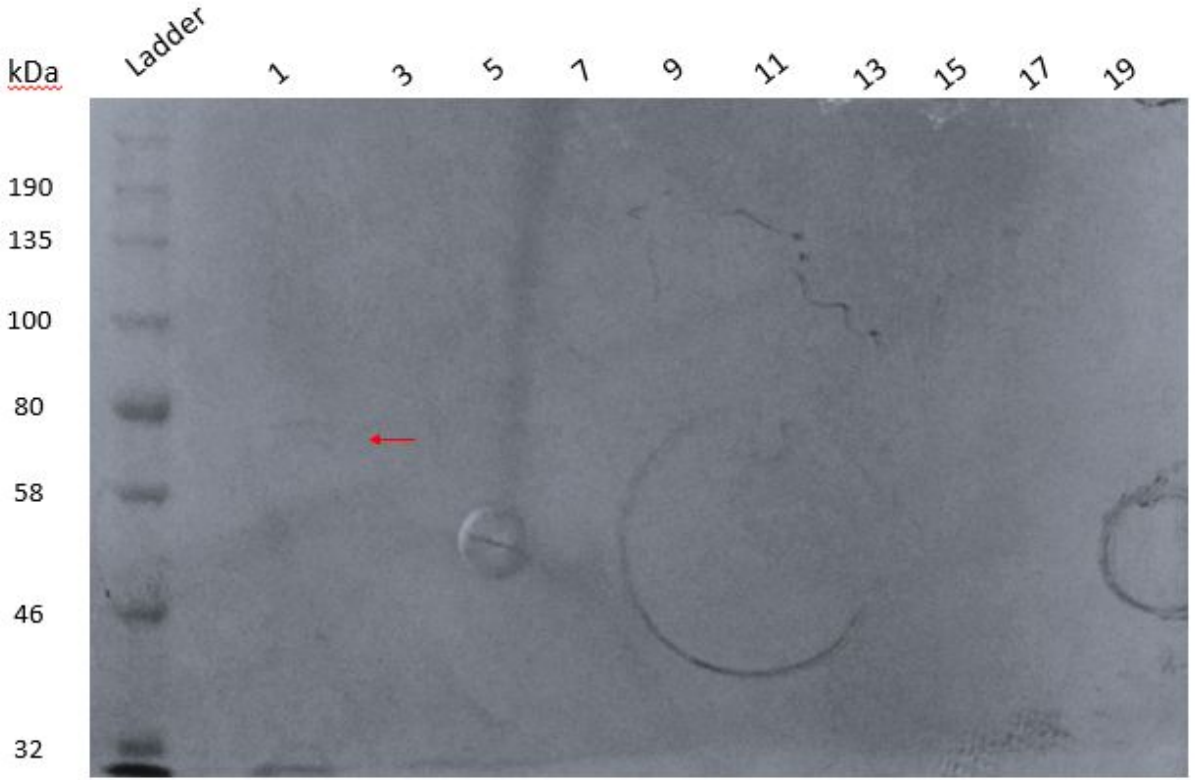
Sample 2-20 : Collected eluted fractions
21- Wash flow through
22- Supernatant flow through



SDS PAGE - Gel 2

Annotations:

Sample 1-19 : Collected eluted fractions



Ni- NTA Purification

Equilibration Buffer (50 mL), Wash Buffer (30 mL), Elution Buffer (50 mL) were prepared according to protocol. But imidazole is not added to wash buffer.

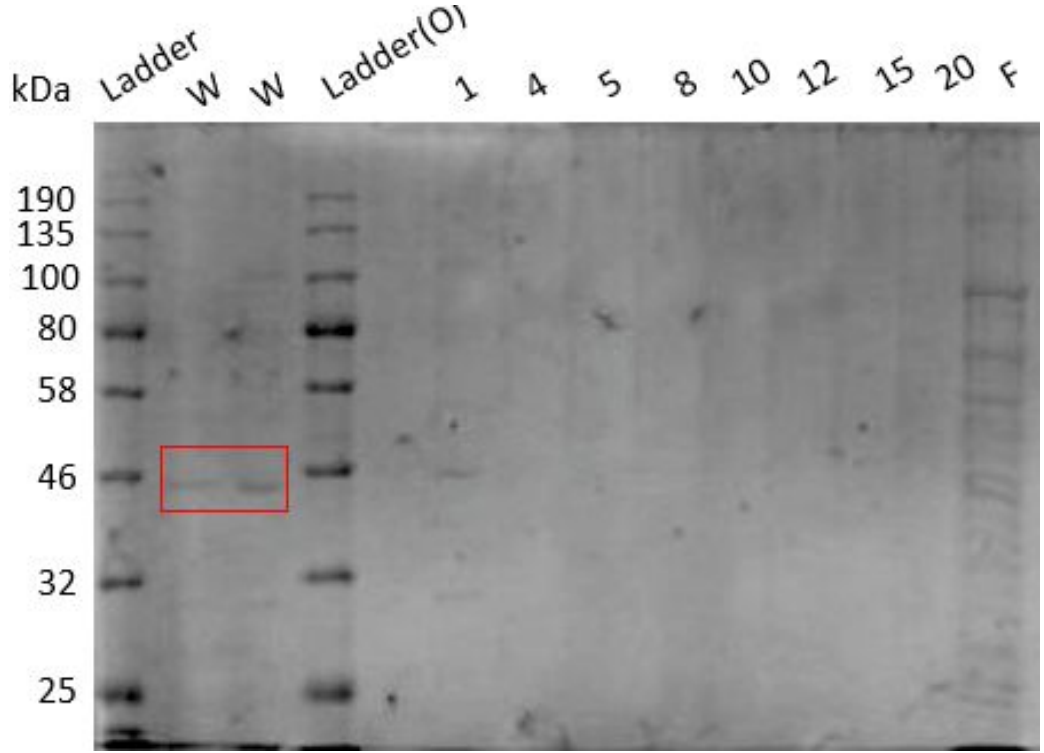
20 mL of equilibration buffer was added to the supernatant flow through from previous Ni-NTA purification in order to dilute the immazidole.

The Supernatant flow through was incubated with Ni-NTA slurry for 1 hour at 4 C on a tube rotator.

5-09-2021

SDS PAGE

Annotations:
W: Wash Through
F: Flow Through



Protein Purification using Qiagen Ni NTA

6-09-2021

1) Inoculation
2) Induction
3) Protein Purification

Lysis and Sonication

7/09/2021

Lysis Buffer

Components	Stock concentration	Working Concentration	Volume required for 40 mL solution
Sodium Phosphate Buffer (pH 7)	0.1 M	50 mM	20 mL
PMSF	1 M	1 mM	40 uL
Sodium Chloride	5 M	0.5 M	4 mL
BME		0.05 % of lysis buffer	20 uL
Glycerol		5 % of lysis buffer	2 mL
Triton X-100	100 %	0.5 % of lysis buffer	200 uL
Protease Inhibitor	1 tablet(EDTA free)	1 tablet (EDTA free)	2 tablet (EDTA free)
Autoclaved Milli Q	To make up 50 mL		7.74 mL

Sonication

On time	30 secs
Off time	30 secs
Amplitude	55%
Total cycles	15
Total time	15 mins

Sonication carried out in pulse mode