

MVMPROA-c008 -p018 6L Large Scale Purification

PAGE24-00563

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Experiment Started:
Projects: **Purification;ASAP**
Related Pages: **PAGE24-00330**
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Expression

Expression done by Nathan in AIM. 6L total, 200g pellet.
MVMPROA-e017

Before cleavage
MH HHHHHH GSGDQEAKPSTEDLGDKKEGEYIKLVIGQDSSEIHFKVKMTTHLKKLKESYCQRQGVPMNSLRFLFE
GQRIADNHTPKELGMEEDVIEVYQEQTGG/////SGLVKMSHPSGDVEACMVQVTCGSMTLNGLWLDNTVWCPR
HVMCPADQLSDPNYDALLISMTNHSFSVQKHIGAPANLRVVGHAMQGTLLKLTVDVANPSTPAYTFTTVKPGAAFS
VLACYNGRPTGTFTVVMRPNYTIKGSFLCGSCGSGVGYTKEGSVINFCYMHQMELANGTHTGSAFDGTMYGAFMD
KQVHQVQLTDKYCSVNVAWLYAAILNGCAWFVKPNRTSVVSFNEWALANQFTEFVGTQSVDM LAVKTGVAIEQL
LYAIQQLYTGFGKQILGSTMLEDEFTPEDVNMQIMGVVMQ

MW: 45381.76
PI: 5.92
E(red): 48360

After cleavage
SGLVKMSHPSGDVEACMVQVTCGSMTLNGLWLDNTVWCPRHVMCPADQLSDPNYDALLISMTNHSFSVQKHIG
APANLRVVGHAMQGTLLKLTVDVANPSTPAYTFTTVKPGAAFSVLACYNGRPTGTFTVVMRPNYTIKGSFLCGSCG
SVGYTKEGSVINFCYMHQMELANGTHTGSAFDGTMYGAFMDKQVHQVQLTDKYCSVNVAWLYAAILNGCAWFV
KPNRTSVVSFNEWALANQFTEFVGTQSVDM LAVKTGVAIEQLLYAIQQLYTGFGKQILGSTMLEDEFTPEDVNMQ
IMGVVMQ

MW: 33330.29
PI: 5.86
E(red): 43890

Purification

Construct IDL MVMPROA-c008
Purification ID: MVMPROA-p018

Buffers:
Lysis buffer - 50 mM HEPES pH 7.4, 500 mM NaCl, 5% glycerol, 0.5mM TCEP

Wash buffer - 50 mM HEPES pH 7.4, 500 mM NaCl, 5% glycerol, 0.5mM TCEP, 30mM imidazole
Elution Buffer - 50 mM HEPES pH 7.4, 500 mM NaCl, 5% glycerol, 0.5mM TCEP, 500mM imidazole
Gel Filtration Buffer (SEC) - **10** mM HEPES pH 7.4, 500 mM NaCl, 5% glycerol, 0.5mM TCEP

1. Lysis buffer supplement with 1:4000 dilution of benzonase, 0.5 mg/mL lysozyme, 1mM MgCl. Incubate for 30min at RT.
2. Sonicated on ice at 50% amplitude for a total of 7-minute sonication time (4 seconds on 12 seconds off) with thick probe.
3. Clarified lysate by centrifugation at 18,000rpm, 4°C for 1 hour. Used JLA16.250 rotor. Supernatant poured into clean beaker.

IMAC

1. Wash and equilibrate 5mL bed volume of Ni Sepharose resin on gravity flow column, first with distilled water, then with wash buffer.
2. Resuspend resin with lysis buffer and add to beaker containing clarified supernatant.
3. Stir gently in cold room for 30mins. Pour only gravity flow column and allow to flow through.
4. Wash resin with 25mL wash buffer twice.
5. Elute with 7.5mL elution buffer, 2 elutions carried out

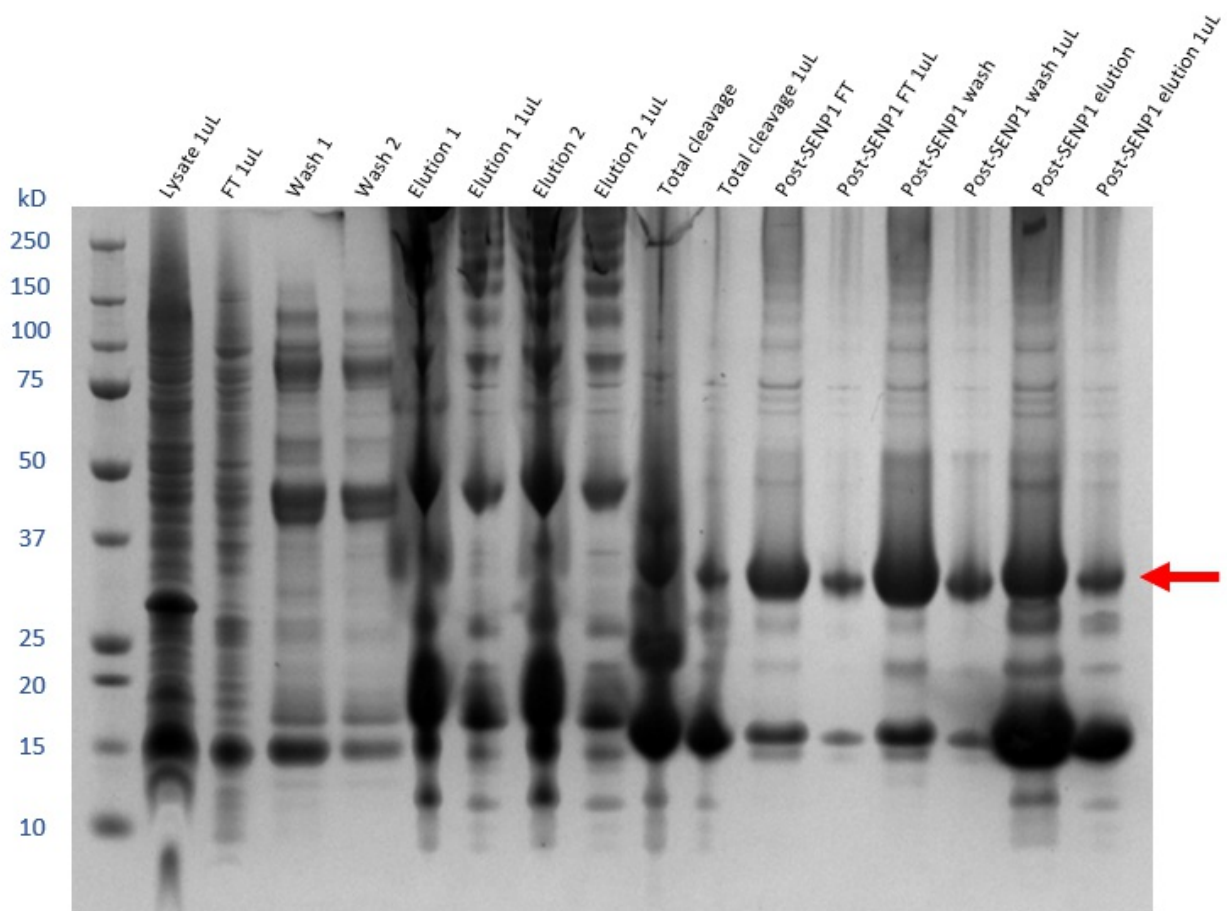
TEV cleavage

1. Desalt elution with HiLoad 10/26 desalting column on ATKA. Desalt into lysis buffer.
2. Pool desalted protein from the fractions
3. Add SENP1 in 1:300 OD ratio. Leave to incubate in cold room.

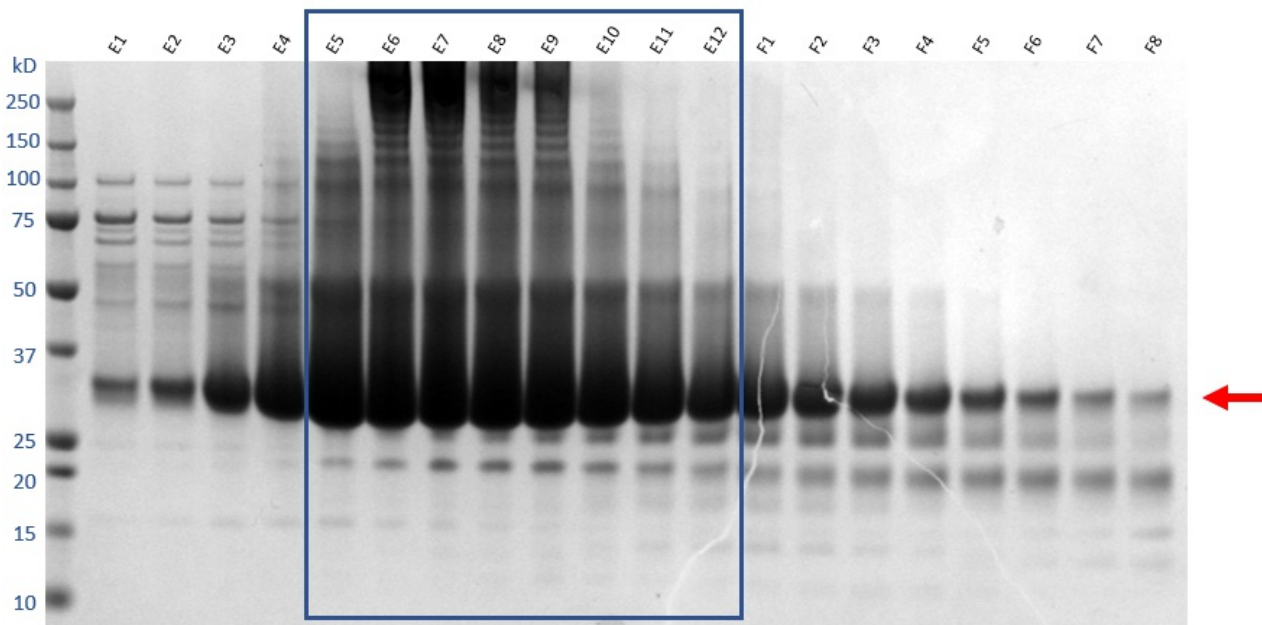
rIMAC and Gel filtration

1. in morning, IMAC resin washed with some lysis buffer to remove imidazole.
2. cleavage mix passed through the resin 1 time (flow rate was a bit slow this time)
3. Wash resin with 10mL wash buffer
4. Elute to see what stuck to the resin
5. rIMAC FT concentrated in 10kDa MWCO Amicon concentrators to final volume of ~5mL
6. Injected onto Superdex s75 16/60 column and run in SEC buffer at 1mL/min
7. After SEC, peaks were analysed by SDS-PAGE

IMAC result



SEC result



Final sample

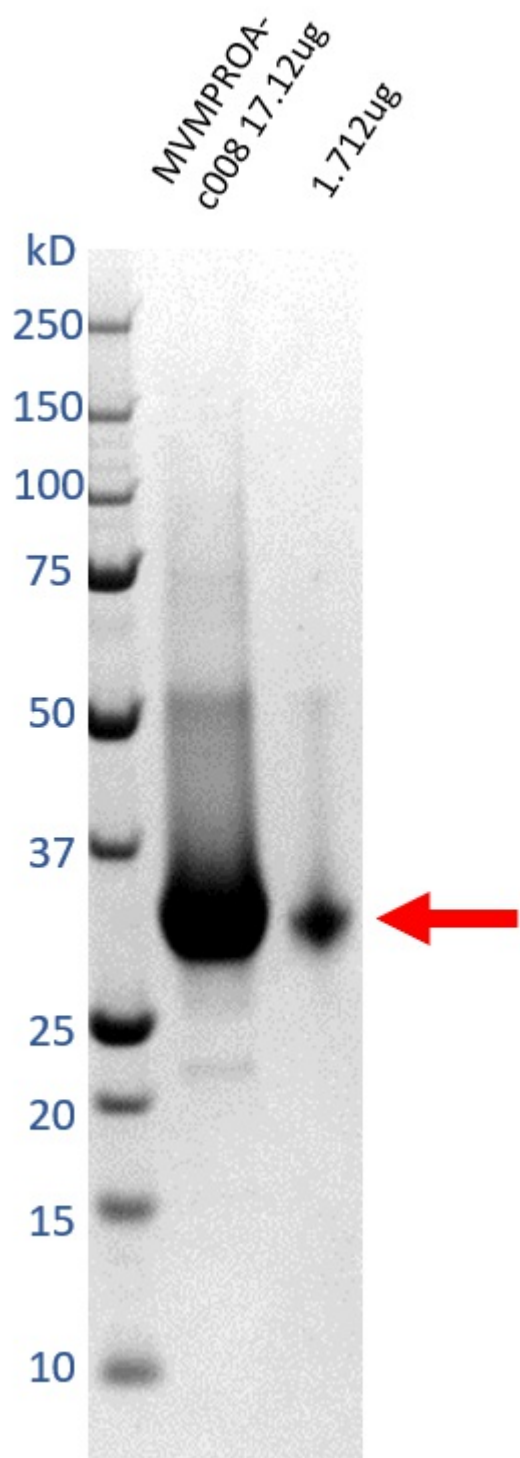
Fractions E5 to E12 were pooled and concentrated in 10kDa MWCO Amicon concentrator.
First used Vivaspin 10kDa, but protein went through entirely and precipitated in the bottom. Probably because the concentrator was broken?
When recovering the sample and attempting to remove aggregate by filtering through syringe, applying pressure on the syringe reversed the aggregation????
Anyways, transferred to the 5mL 10kDa Amicon concentrators to continue
Could only get to 17.12 mg/mL before protein started to aggregate in the concentrator.

Final sample:

17.12 mg/mL
62*50uL + 15uL

Final yield: 53.3 mg/mL

Final sample



MS

