CVMPROA-
c001 p003 SARSMpro in pNIC transformation, expression and purification

PAGE24-00585

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Date Started: **2024-Mar-22**
Experiment Started:
Projects: **Expression**;**Purification**;**ASAP**
Related Pages: **PAGE23-00603**;**PAGE23-00782**
Referenced by:
Tags:

Transformation

Plasmid was purchased from Twist and dissolved in 10uL of nuclease-free water.
Transformation carried out per the standard protocol.

CVMPROA-c001

Expression

22/03/2024

6x1L Invitrogen TB + Kan + 4mL glycerol after autoclave + Kan50
1mL of ON from transformation was used to inoculate per L of TB. Grown at 37oC 200rpm until OD~1.8, induced with 0.5mM IPTG
Temperature reduced to 18oC and speed to 180rpm. Grown overnight.

Harvest next day. Total pellet weight = 88g

IMAC, cleavage and rIMAC

MGSSHHHHHHGSDSEVNQEAKPEVKPEVKPETHINLKVSDGSSEIFFKIKKTTPLRRLMEAFAKRQGKEMDSLRFLYDGIRIQADQTPEDLDMEDNDIIEAHREQIGGSGFRKMAFPSGKVEGCMVQVTCGTTTLNGLWLDDVVYCPRHVICTSEDMLNPNYEDLLIRKSNHNFLVQAGNVQLRVIGHSMQNCVLKLKVDTANPKTPKYKFVRIQPGQTFSVLACYNGSPSGVYQCAMRPNFTIKGSFLNGSCGSVGFNIDYDCVSFCYMHHMELPTGVHAGTDLEGNFYGPFVDRQTAQAAGTDTTITVNVLAWLYAAVINGDRWFLNRFTTTLNDFNLVAMKYNYEPLTQDHVDILGPLSAQTGIAVLDMCASLKELLQNGMNGRTILGSALLEDEFTPFDVVRQCSGVTFQ

Purification ID: CVMPROA-p001 (labelled p003 on bag)

Buffers:
Lysis buffer - 50 mM HEPES pH 7.4, 150 mM NaCl, 5% glycerol, 0.5mM TCEP

Wash buffer - 50 mM HEPES pH 7.4, 150 mM NaCl, 5% glycerol, 0.5mM TCEP
Elution Buffer - 50 mM HEPES pH 7.4, 150 mM NaCl, 5% glycerol, 0.5mM TCEP, 500mM imidazole
Gel Filtration Buffer (SEC) - 50 mM HEPES pH 7.4, 150 mM NaCl, 5% glycerol, 0.5mM TCEP

no imidazole in binding and wash step due to previous difficulties with target not binding to IMAC resin

1. Lysis buffer supplement with 1:4000 dilution of benzonase, 0.5 mg/mL lysozyme. 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 equlibrate 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 though.
4. Wash resin with 25mL wash buffer twice.
5. Elute with 7.5mL elution buffer, 2 elutions carried out

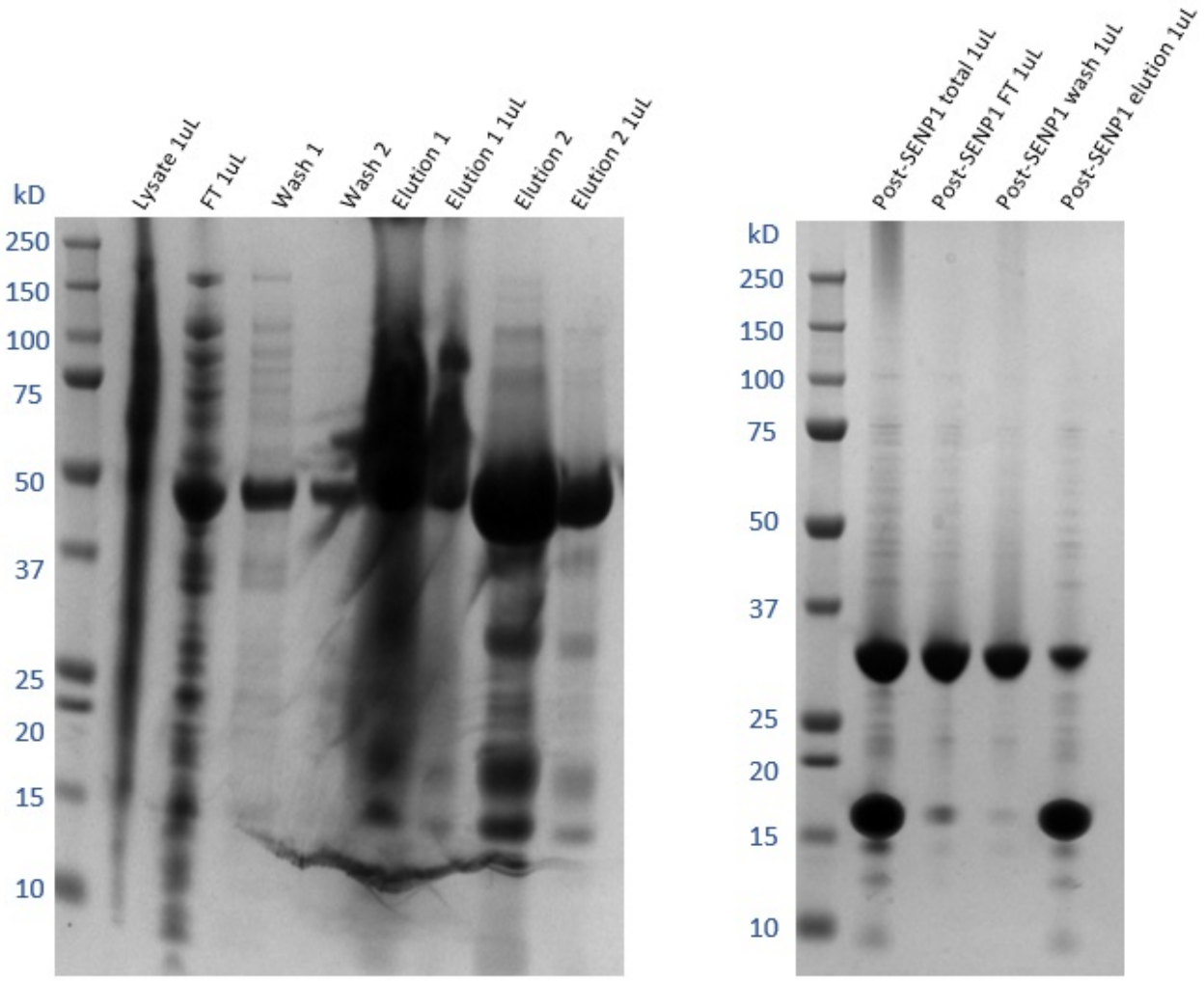
TEV cleavage

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.

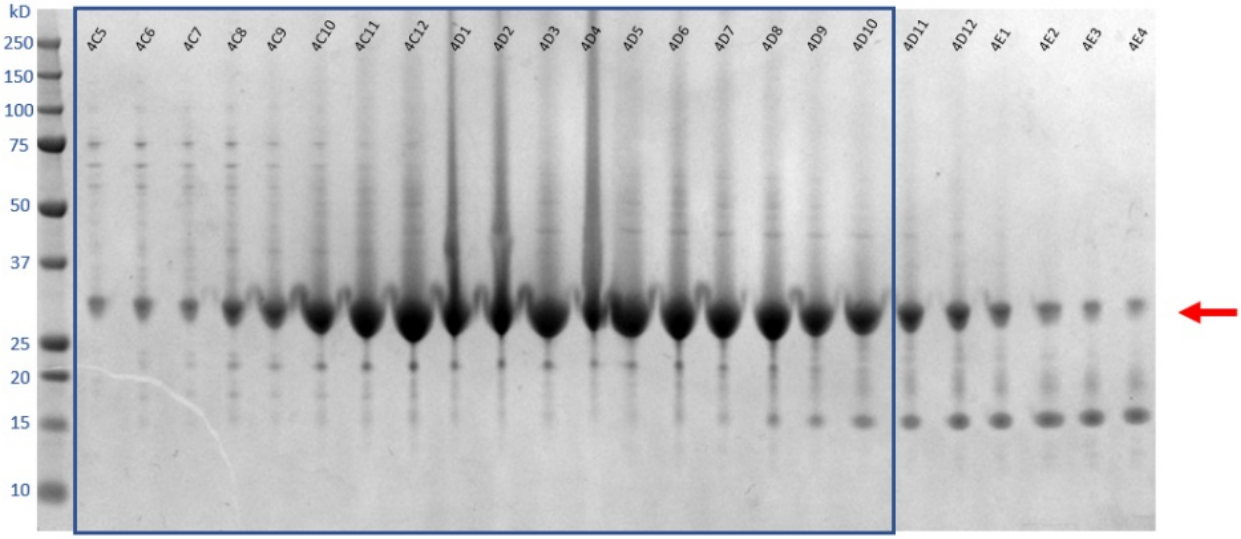
rIMAC and Gel filtration

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 yellow vivaspin concentrators to final volume of ~5mL
6. Injected onto Superdex s200 16/60 column and run in SEC buffer at 1mL/min
7. After SEC, peaks were analysed by SDS-PAGE

IMAC results


kD
250
150
100
75
50
37
25
20
15
10
Lysate 1uL
FT 1uL
Wash 1
Wash 2
Elution 1
Elution 1 1uL
Elution 2
Elution 2 1uL
kD
250
150
100
75
50
37
25
20
15
10
Post-SENP1 total 1uL
Post-SENP1 FT 1uL
Post-SENP1 wash 1uL
Post-SENP1 elution 1uL

SEC result


kD
250
150
100
75
50
37
25
20
15
10
4C5
4C6
4C7
4C8
4C9
4C10
4C11
4C12
4D1
4D2
4D3
4D4
4D5
4D6
4D7
4D8
4D9
4D10
4D11
4D12
4E1
4E2
4E3
4E4

Final sample

Fraction 4C5 - 4D10 were pooled and concentrated in the yellow vivaspin 10kDa MWCO concentrators

Final concentration: 26.96 mg/mL, 47*100 + 50 uL
Total yield: 128.1 mg

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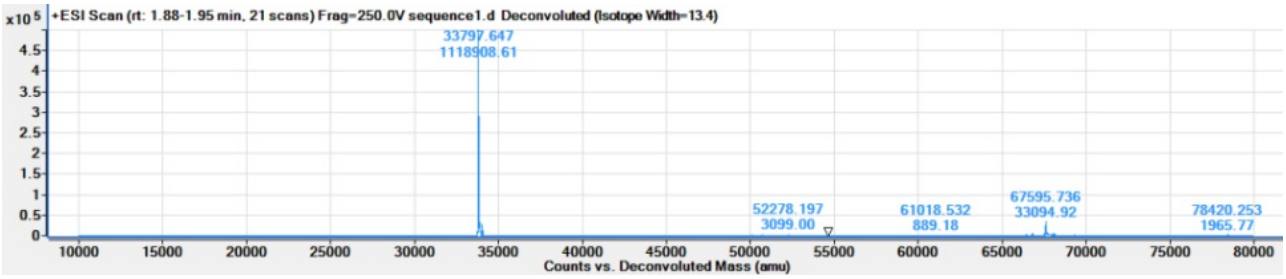

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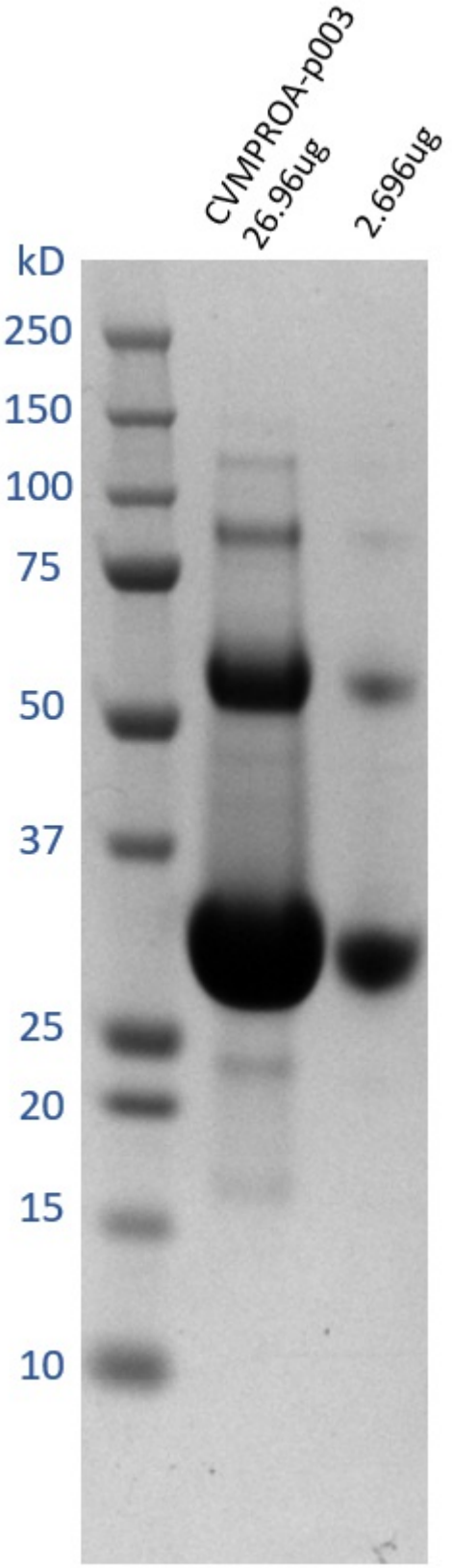
final sample


CVMPROA-p003
26.96ug
2.696ug
kD
250
150
100
75
50
37
25
20
15
10