

New Zika RdRp AIM+LB expression and purification test

PAGE24-00544

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Projects: **Expression;Purification;ASAP**

Related Pages: **PAGE24-00136**

Referenced by:

Tags:

Expression

3L each. AIM pellet was quite small but still bigger than LB.

Did not weigh :(

Grew in 3L each AIM (not Mike's) and LB.

For LB: grew at 37oC 250PRM until OD~0.8. Induced with 0.5mM IPTG like orginal. Induced overnight at 18oC and harvested next morning.

For AIM: grew at 37oC 250RPM until OD~2. Temperature reduced to 18oC, 180RPM shaking for 27 hrs. Harvested afternoon of second day. (2 day induction)

Harvested 4000xg 4oC 30min.

IMAC cleavage and rIMAC

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, 20mM imidazole

Elution Buffer - 50 mM HEPES pH 7.4, 500 mM NaCl, 5% glycerol, 0.5mM TCEP, 300mM imidazole

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

1. Lysis buffer supplement with 1:4000 dilution of benzonase, 0.5mg/mL lysozyme, 1mM MgCl. Incubate for 30min at RT.
2. Sonicated on ice at 40% amplitude for a total of 3-minute sonication time (4 seconds on 12 seconds off) with medium probe.
3. Clarified lysate by centrifugation at 18,000rpm, 4°C for 1 hour. Supernatant poured into fresh centrifuge bottle.

IMAC (for each expression)

1. Wash and equilibrate 1mL bed volume of Ni Sepharose resin on gravity flow column, first with distilled water, then with wash buffer.
2. Put stopper on column. Re-suspend resin with small volumes of the lysis buffer, and pour into lysate. Rotate in cold room for 10mins.
3. Pour mixture back onto gravity flow column and let flow through.
4. Wash resin with 10 mL wash buffer twice.
5. Elute with 5mL elution buffer, 2 elutions carried out

AIM E1: 4.12 mg/mL

AIM E2: 1.93 mg/mL

LB E1: 2.67 mg/mL

LB E2: 0.81 mg/mL

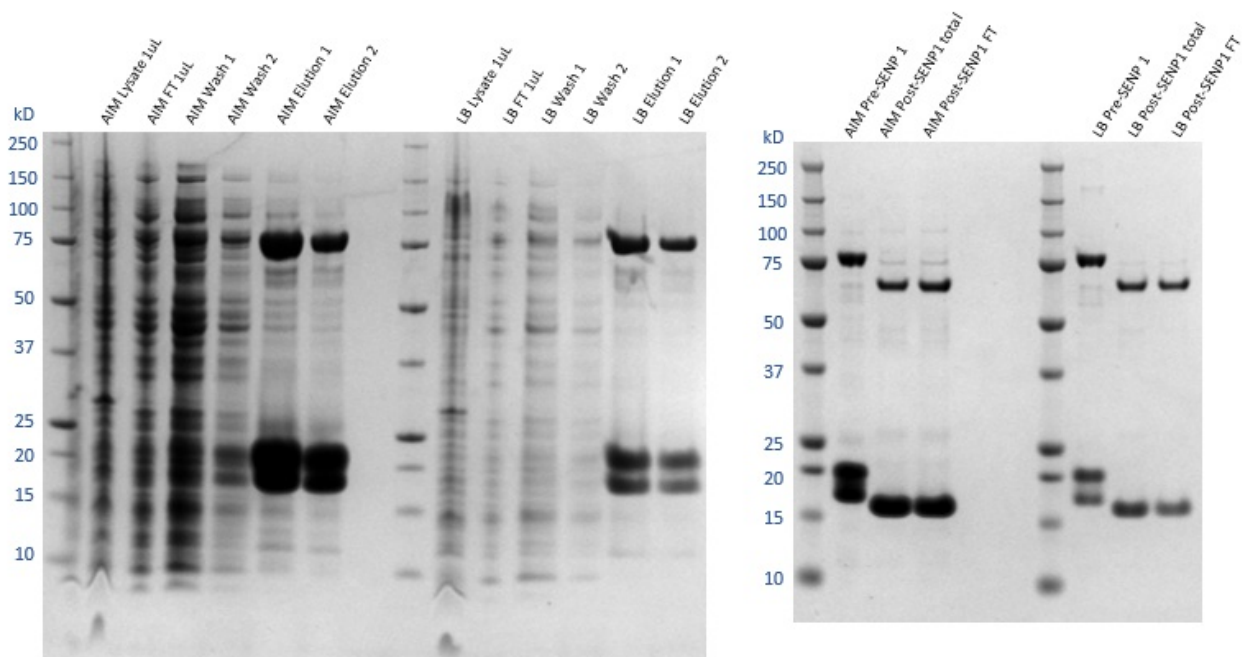
SENP1 cleavage

1. Diluted to 50mL (final imidazole concentration 30mM) with lysis buffer.
2. Add SENP1 in 1:300 OD ratio. Leave to incubate in cold room.

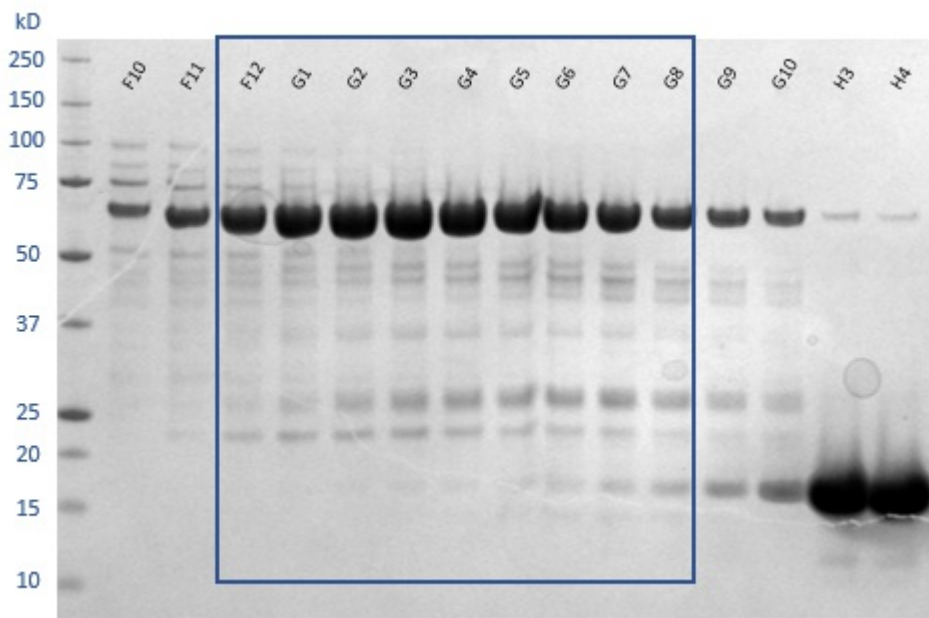
rIMAC and Gel filtration

1. in morning, used resin washed with some lysis buffer to remove imidazole.
2. cleavage mix passed through the resin 3 times
AT THIS POINT THE rIMAC FT FROM THE TWO EXPRESSIONS WERE POOLED
3. rIMAC FT concentrated in 30kDa MWCO Vivaspin concentrators to final volume of ~2mL
4. Injected onto Superdex s200 16/600 column and run in SEC buffer at 1mL/min
5. After SEC, peaks were analysed by SDS-PAGE

IMAC cleavage and rIMAC results



SEC result



Final sample

Fractions F12 to G8 were pooled and concentrated in 10kDa MWCO Amicon concentrator.

Final sample:

7.88 mg/mL
12*50uL + 10uL

Final yield: 4.81 mg/mL

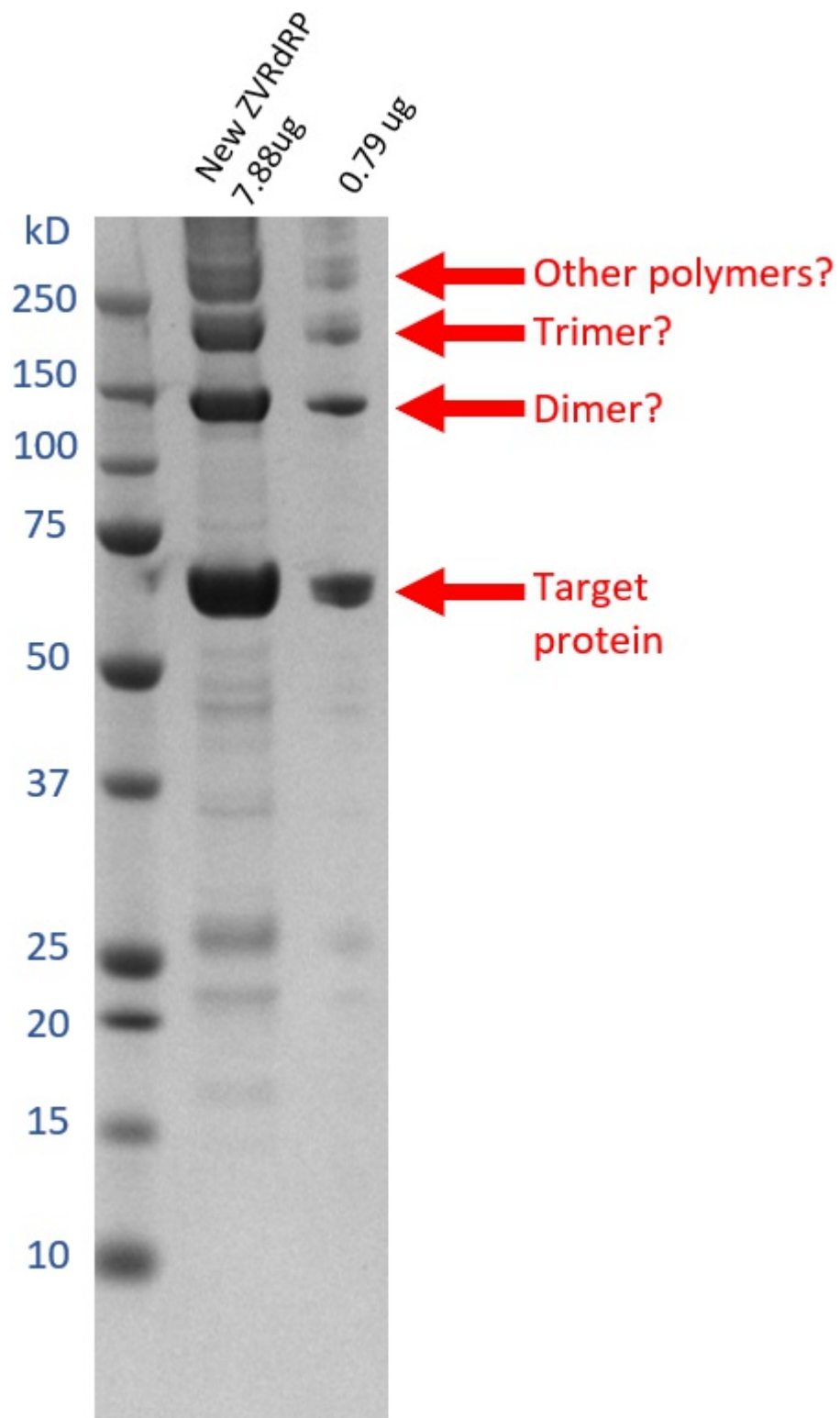
this is around 4x what we usually get from the previous construct

Anu said she gets 5-6mg per 2L prep, so this is still not the best. Next time can try Mike's AIM media which always tends to work better.

- Ran final sample gel a week later and saw some bands that look like polymeric forms of ZVRdRP? These higher bands were not there during SEC so should not be contaminants.

When have time - can boil the sample for longer to see if that breaks up the polymers

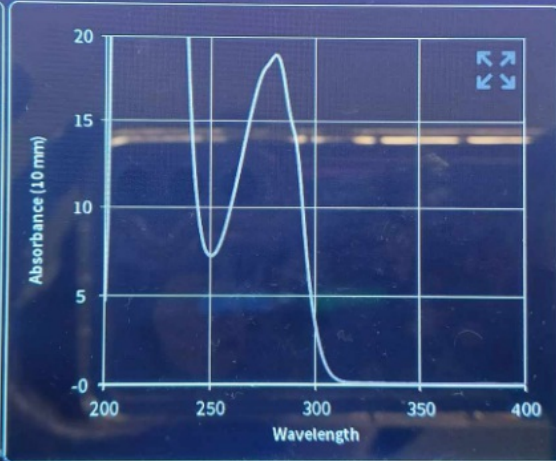
Final sample



Nanodrop

Settings menu with icons for sliders, clipboard, graph, and list.

Custom 0.423
Volume 1 μ l - 2 μ l
Wavelength 280 nm
Units mg/ml
Background Correction
Air Bubble Recognition
Dye Label
Manual Dilution



Concentration
7.8801
mg/ml
A260/A280 0.547
A280 (10 mm path) 18.67

Change to Cuvette enter sample name BLANK AUTO SAMPLE SAMPLE