Expression and Purification of Zika NS5 RDRP Trial 4

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Projects: **Expression**;**Purification**
Related Pages: **PAGE22-01851**;**PAGE22-02177**;**PAGE23-00163**
Referenced by:
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Background

Taking previous purification trials and optimisations into account, the Zika NS5 RDRP appears to be degrading after the cleavage of its His-Thioredoxin tag. The previous purification resulted in issues during the cleavage and reverse IMAC stage of the process. This trial will utilise a rapid IMAC process with minimal incubation time and add Mg^{2+} to the cleavage reaction to try and stabilise the protein upon tag removal.

Protein Expression

A starter culture (3 x 30 mL) using 50 µg/mL of Kanamycin was grown at 37 °C and 250 RPM overnight by Kory Wang, from her personal glycerol stock. The following day the starter cultures (OD_{600} - 1.8) were pooled and gently mixed, 6 x 1L of fresh LB Broth (Miller formulation) was inoculated (1030 hours) with 4.5 mL of pooled starter culture, with 50 µg/mL of Kanamycin.

The 6 x 1L cultures were grown at 37 °C and 250 RPM, until they reached an absorbance of (OD_{600} - 0.7) (1430 hours) and then subsequently cooled at 18 °C with no mixing for 10 minutes. Cells were induced with IPTG (500 µM) and left overnight to express at 18 °C, 120 RPM.

The following morning the cells had reached (OD_{600} - 1.2) and were harvested by centrifugation at 5000 x g for 20 minutes at 4 °C. Final wet pellet weight was 18.2 g.

Buffer List

Lysis Buffer

50 mM HEPES pH 7.5
500 mM NaCl
25 mM Imidazole
5 % (v/v) Glycerol
0.5 mM TCEP-HCl

Supplemented with: 0.5 mg/mL Lysozyme, 1 X cOmplete™ EDTA free Protease Inhibitor, 2 mM $MgCl_2$ and Benzonase (1:4000 dilution).

Wash Buffer

50 mM HEPES pH 7.5
500 mM NaCl
25 mM Imidazole

5 % (v/v) Glycerol
0.5 mM TCEP-HCl

Elution Buffer

50 mM HEPES pH 7.5
500 mM NaCl
500 mM Imidazole
5 % (v/v) Glycerol
0.5 mM TCEP-HCl

TEV Cleavage Buffer

50 mM HEPES pH 7.5
500 mM NaCl
5 % (v/v) Glycerol
2 mM $MgCl_2$
0.5 mM TCEP-HCl

Gel Filtration Buffer

50 mM HEPES pH 7.5
500 mM NaCl
5 % (v/v) Glycerol
0.5 mM TCEP-HCl

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Upon harvesting cells, the pellet was immediately incubated with Lysis buffer and left to mix on a magnetic stirrer in the cold room (4 °C). After the pellet was fully homogenised (1 hour), the lysate was sonicated (35 % amplitude, 4 seconds on, 4 seconds off, 10 minutes on time with the 0.75 inch sonication probe). The lysate was clarified by centrifugation (JLA 25.50 rotor, 4 °C for 20 minutes at 50000 x g) and the resulting supernatant was incubated with Nickel Sepharose 6 Fast Flow (Cytiva) affinity resin for 30 minutes on a rotator.

The supernatant was poured onto a clean and equilibrated gravity flow column. The resin was washed with Wash Buffer and eluted with 3 x 5 mL of Elution Buffer. The elutions were pooled and immediately buffer exchanged into TEV Cleavage Buffer using a Hiprep 26/10 Desalting column (Cytiva).

Fresh TEV (new batch) was added to the sample at a ratio of 1:20 TEV/Protein:

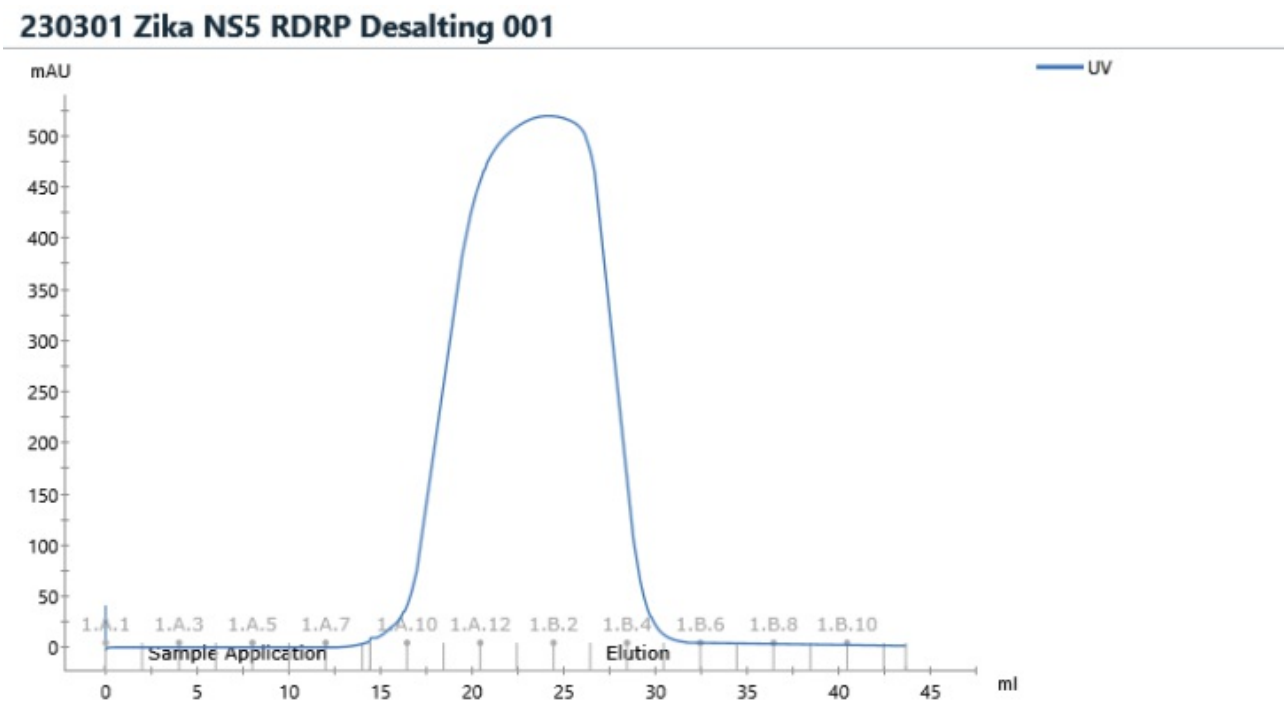
Protein concentration: 1.47 mg/mL in 16 mL - 23.5 mg total yield (1 mg of TEV added)

The TEV cleavage reaction was left at room temperature (20 °C) for 1 hour and immediately poured onto fresh equilibrated Nickel Sepharose 6 Fast Flow resin in a gravity column. The sample was not incubated in the resin and allowed to pass staright through to prevent any potential precipitation, which was previously observed. The sample was collected and reapplied to the column for a total of 3 passes to capture as much uncleaved protein as possible, while reducing incubation time. Precipitation was once again visible, once again indicating that the protein is unstable and degrading after tag cleavage.

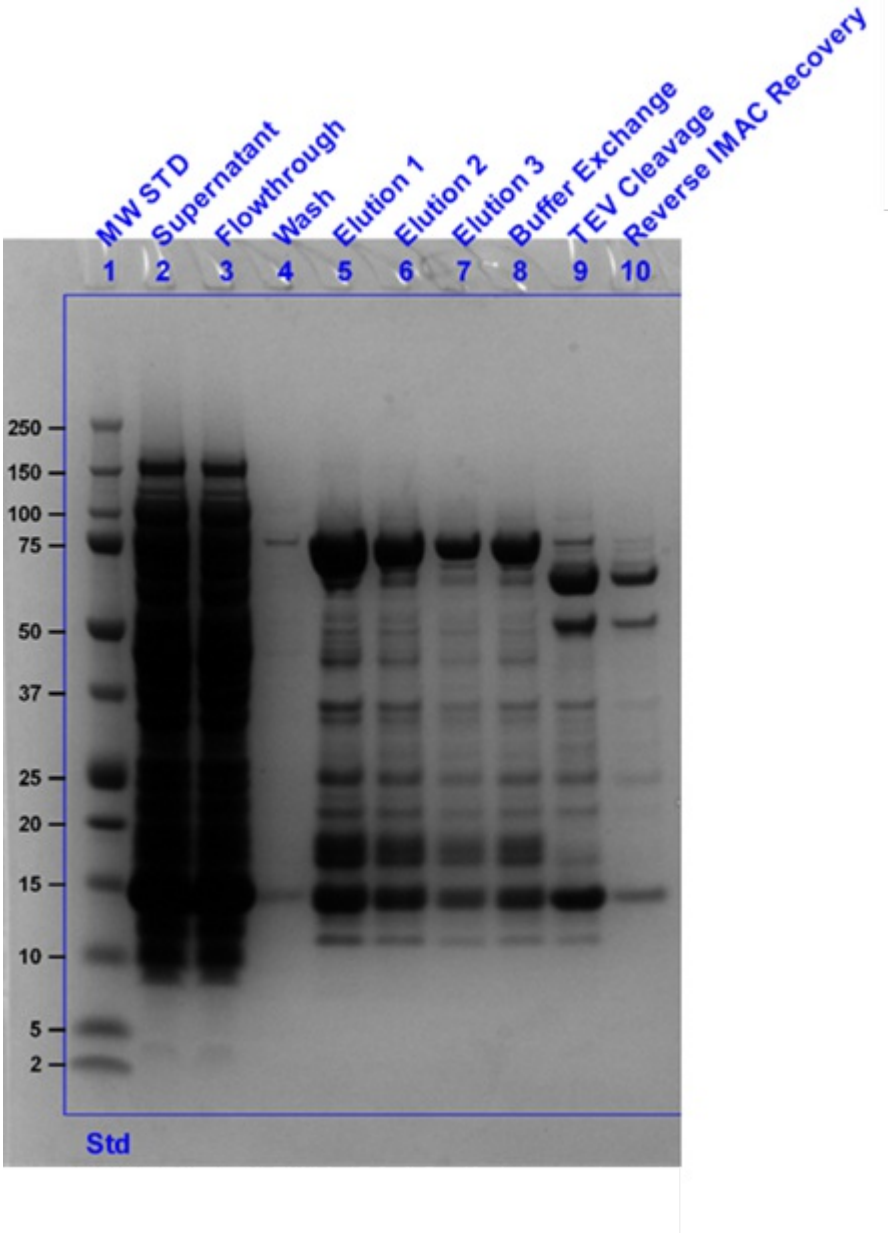
The resulting flowthrough was concentrated to 1 mL and injected into an equilibrated HiLoad 16/60 Superdex 200 gel filtration and the purest fractions corresponding to the target protein (MW = 69 kDa) were concentrated to 6.1 mg/mL and flash frozen in liquid nitrogen.

Final yield after concentration = 3 x 30 uL at 6.1 mg/mL = 0.55 mg

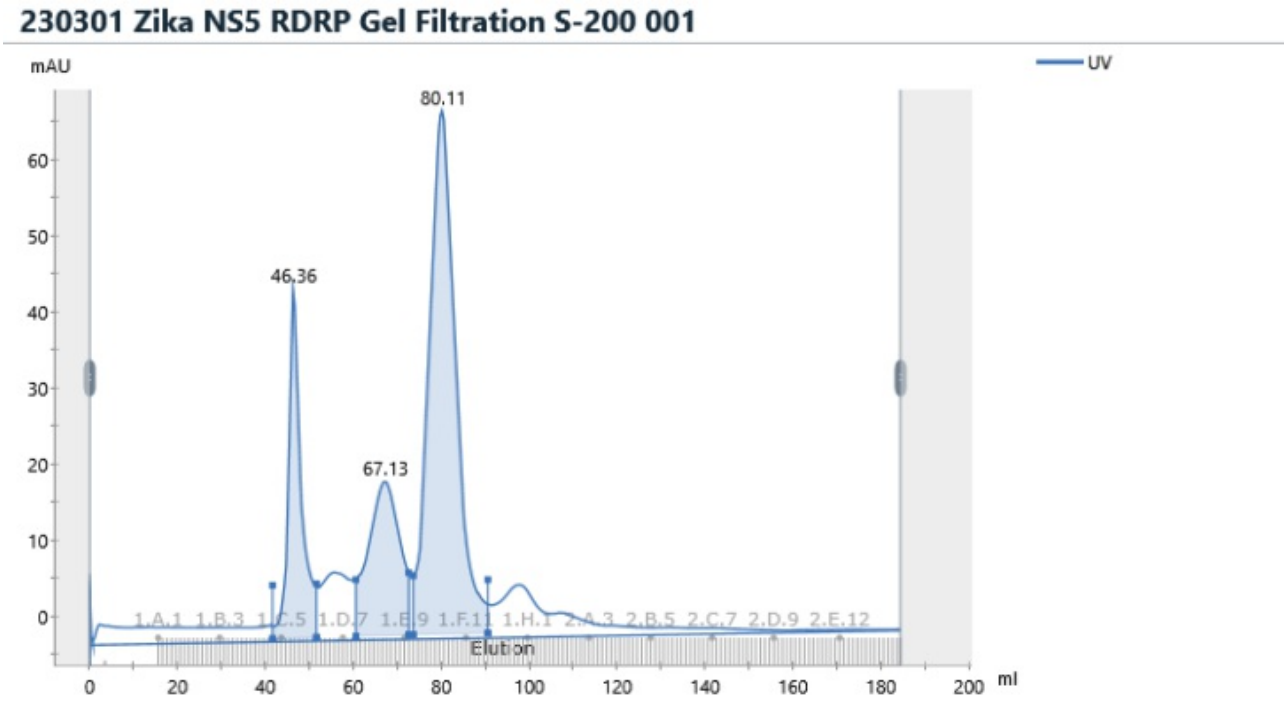
HiPrep 26/10 Desalting Buffer Exchange Chromatogram



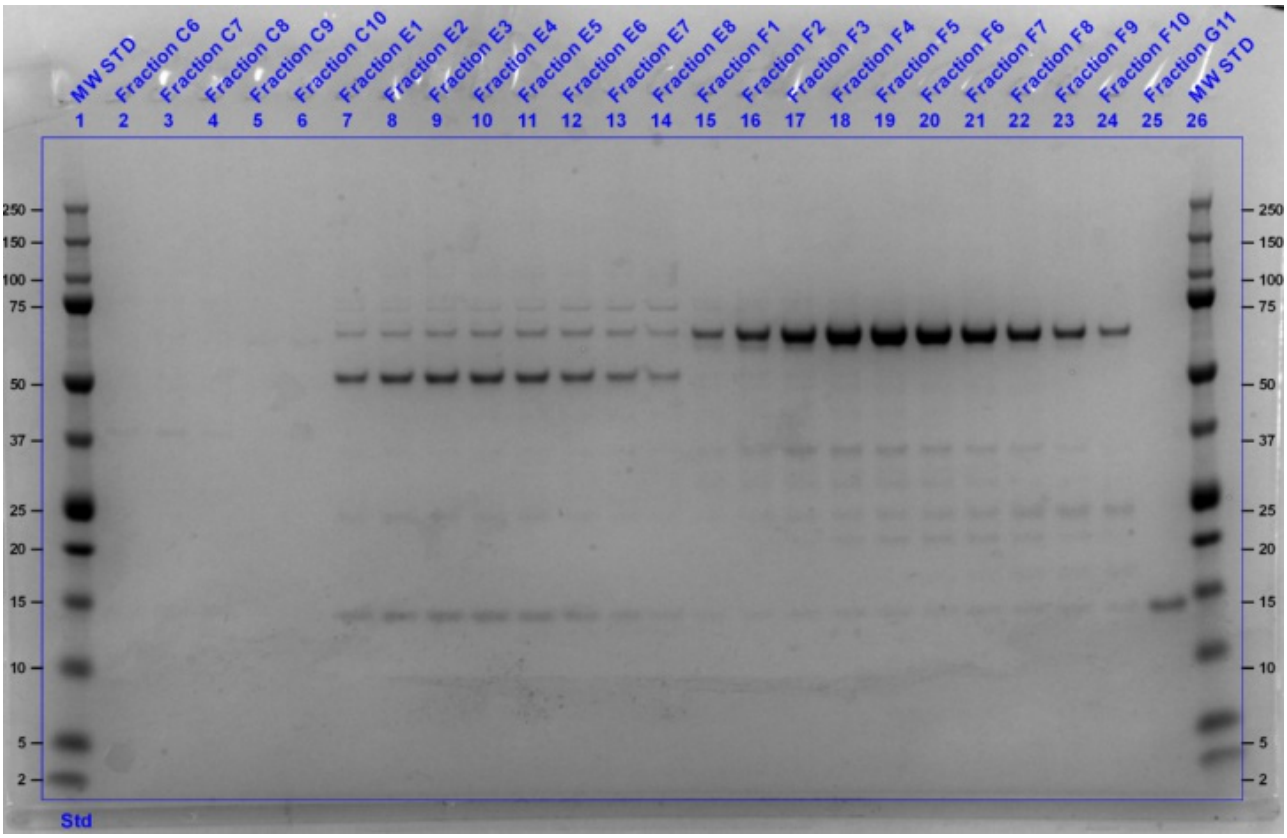
SDS-PAGE of Purification and TEV Cleavage



HiLoad 16/60 Superdex 200 Gel Filtration Chromatogram



SDS-PAGE of Gel Filtration Fractions



Concluding Thoughts

Overall the purity of the gel filtration fractions look very good and the TEV cleavage seems greatly improved with the latest batch of TEV protease, however overall yield is still low and the protein is still unstable and

degrading.

Due to the consistently observed instability of this protein, rapid purification appears to be the main determining factor in achieving a high purity. Other than bulking up future expression, another option could be autoinduction to generate a large pellet, that leads to greater yields. Previous expressions with TB had issues, so possibly a small 1 - 2 L trial would be enough to rule this option out.