

JEDI Program/JDI01

Compound Screening for SARS-CoV-2 Proteins Using MST/Dianthus

Reconstitution and purification of the RNA-dependent RNA Polymerase complex

June 24, 2021

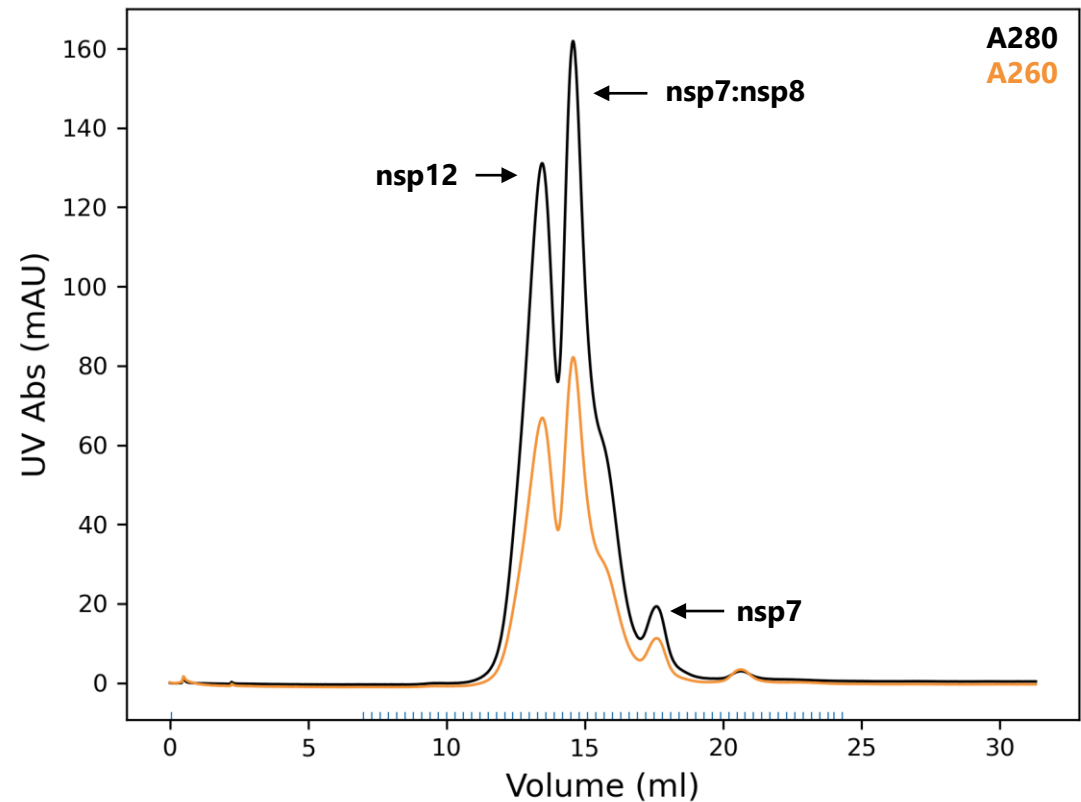


Purification summary - nsp12:nsp8:nsp7 (DVT1:DZT1:DZU1)

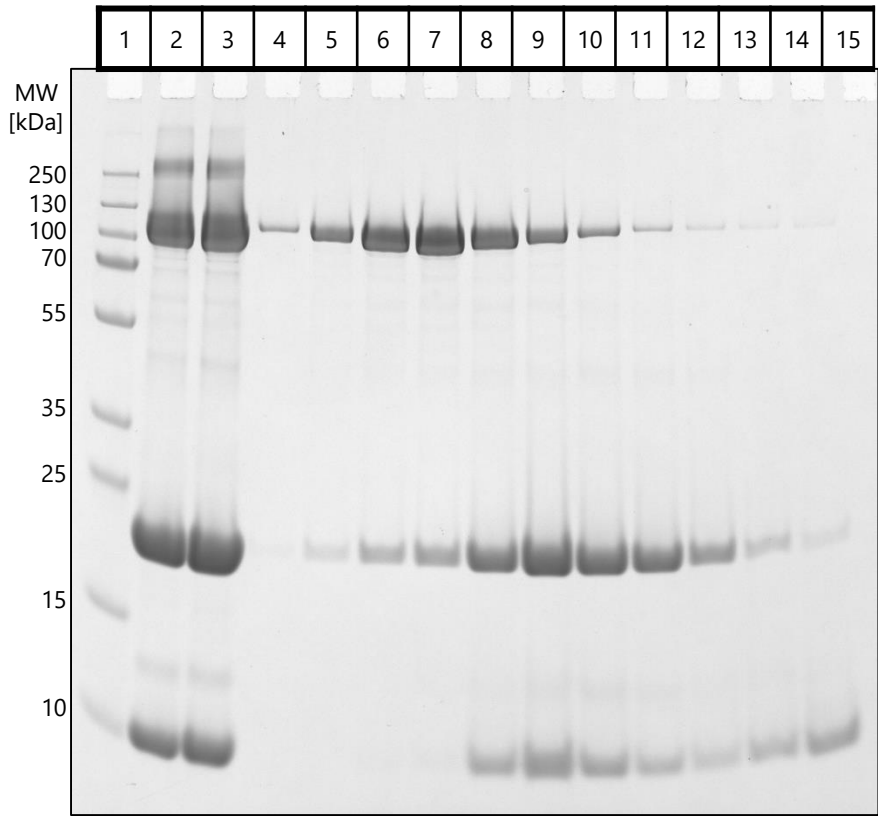
- The complex of nsp12:nsp8:nsp7 (DVT1:DZT1:DZU1) was reconstituted from its individual subunits
 - The subunits were purified individually according to Hillen et al., Nature, 2020
 - The subunits were mixed at an excess of nsp8 and nsp7, dialyzed in the storage buffer and purified by semi-preparative SEC
 - The storage buffer was chosen according to Madru et al., PLOS ONE, 2021 (<https://doi.org/10.1371/journal.pone.0250610>)
 - Difference to buffer in previous purification: NaCl concentration increased to 300 mM and pH changed from 8.0 to 7.4
- SDS-PAGE analysis and the chromatogram of the semi-preparative SEC show that the subunits do not form a stable complex under these conditions but elute individually from the SEC column
 - No intact complex could be formed in the buffer described by Madru et al. PLOS ONE, 2021
- In total, three experiments have been conducted to reconstitute the SARS-CoV2 polymerase complex under the following conditions:
 - Low salt (100 mM) without RNA scaffold
 - Low salt (100 mM) with RNA scaffold
 - Medium salt (300 mM) without RNA scaffold
 - A SEC-stable complex could only be formed in presence of RNA at low salt but this sample is sensitive to temperature and hence not suitable for biophysical experiments

Semi-preparative SEC - nsp12:nsp8:nsp7 (DVT1:DZT1:DZU1)

Chromatogram / CHR1 SEC



SDS-PAGE / Coomassie stained



Lane	Sample
1	Marker: PageRuler Plus
2	Pooled subunits
3	Pooled subunits after dialysis
2-15	SEC elution

- Individual subunits elute individually from the SEC and do not form a stable complex

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