

JEDI Program/JDI01

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

Quality control SARS-CoV-2 polymerase complex (nsp12:nsp8:nsp7)

July 15, 2021



QC summary - nsp12:nsp8:nsp7 PD15376

- The SARS-CoV-2 polymerase complex (nsp12:nsp8:nsp7) was analyzed by standard quality control comprising SDS-PAGE and analytical SEC
- SDS-PAGE shows a pure protein sample with clear bands for nsp12 and nsp8 but no signal for nsp7
→ nsp7 is underrepresented or not present in final sample
- Analytical SEC performed at room temperature shows two peaks indicating dissociation of the complex and separate elution of nsp12 and nsp8
→ The complex is not stable under the tested condition and might be temperature-sensitive
- Taken together, the quality control demonstrates that the sample does not contain intact, trimeric SARS-CoV2 polymerase complex suitable for biophysical experiments
- Optional next step: MS analysis to analyze integrity of the individual subunits and identify potential traces of nsp7 in the sample

Protein data sheet - nsp12:nsp8:nsp7 PD15376

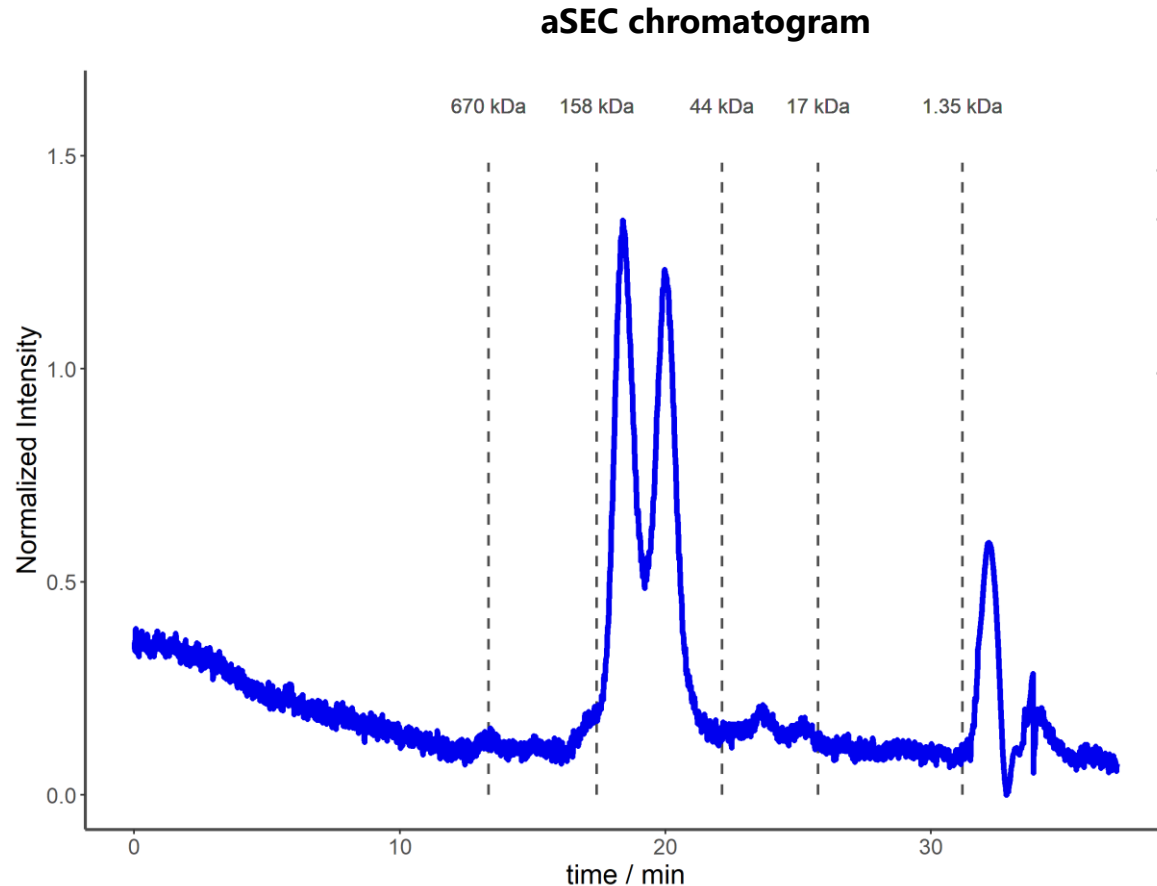
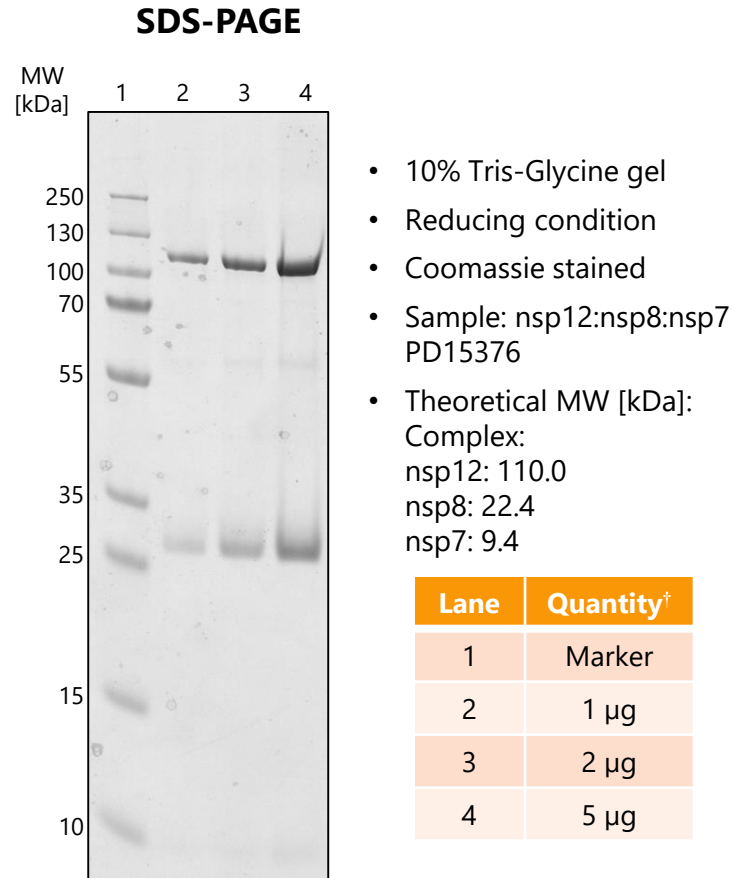


Protein: nsp12:nsp8:nsp7 (Uniprot ID: P0DTD1)
Construct ID: n.a.
Lot-ID: PD15376

Protein Purification	Concentration (A280, mg/mL) [†]	Aliquot size (μL)	A260/A280	Modification / Ligand	Tag
	3.1	50	n.a.	none	N-terminal on nsp12: His ₁₄ -TEV
	Expression system	Purification steps	Storage buffer		
	E. coli	n.a.	10 mM Hepes/NaOH, 150 mM NaCl, 0.5mM MgCl ₂ , 50% Glycerol, pH 8.0		
Quality control *	Analytical SEC	Purity based on SDS-PAGE	Intact mass (Da)	Peptide mass fingerprinting	
	two peaks	>90%	n.a.	n.a.	
Protein parameters	Theoretical MW (Da)	Theoretical extinction coefficient (= 1 g/L)	Construct boundaries	Mutations	Theoretical pI
	nsp12: 109980.7 nsp8: 22429.7 nsp7: 9371.0	1.14	n.a.	n.a.	6.8
	Amino acid sequence				
	nsp12: MGKHHHSHGHHHTGHHHSHGSHHHTSSASTGSADAQSFLNRVCVSAARLTPCGTGTSTDVVYRAFDIYNDKVAGFAKFLKTNCCRFQEKDEDDNLIDSYFVVKRHTFSNYQHEETIYNLLKDCPAVAKHDFKFRIDGDMVPHISRQRLTKYTMADLVYALRHFDEGNCD TLKEILVTYNCCDDDYFNKKDWYDFVENPDILRVYANLGERVRQALKTVQFCDAMRNAGIVGLTLDNQDLNGNWDYDFGDFIQTPGSGVVPVDSYSSLMPILTLTRALTAESHVDTDLTKPYIKWDLKDYDFTEERLKLFDRIYKYWDQTYHPNCVNCDDRCILHCANFNV LFSTVFPTSFGLPLVRKIFVDGVPFVYSTGYHFRELGVVHNQDVNLHSSRLSFKELLVYAADPAMHAASGNLLLDKRTTCFSAALTNNVAFQTVKPGNFNKDFYDFAVSKGFFKEGSSVELKHFFFAQDGNAAISDYDYRYNLPTMCDIRQLLFVVEVDKYFDCYDGGCINAN QVIVNNLDKSAGFPFNKGKARLYYDSMSYEDQDALFAYTKRNVIPITITQMNLKYAISAKNRARTVAGVSICSTMNRQFHQKLLKSIAATRGATVVGTSKFYGGWVHNMMLKTVYSDVENPHLMGWDPKCDRAMPNMLRIMASLVLARKHTTCCSLSHRFYRLANCAQVL SEMVMCGGSLYVKPGGTSSGDATTAYANSVFNICQAVTANVNALLSTDGNGKIADKYVRNLQHRLYECLYRNRDVTDFVNEFYAYLRKHFSMMILSDDAVVCFNSTYASQGLVASIKNFKSVLYYQNNVFMSEAKCWTETDLTKGPHFCSQHTMLVKQGDDYVYLPYPDPS RILGAGCFVDDIVKTDGTLMIERFVSLAIDAYPLTKHPNQEYADVFFHLYLQYIRKLHDELTHMLDMYSVMLTNDNTSRYWEPEFYEAMYPHTVL nsp8: MTGDGSAIAEFSSLPYAAAFATAQEAYEQAVANGDSEVVLLKKLKSLSNVAKSEFDRDAAMQRKLEKMADQAMTQMYKQARSEDKRAKVTSAMQTMLFTMLRKLNDALNIIINNARDGCVPLNIHPLTTAAKLMVVIPDYNTYKNTCDGTTFTYASALWEIQQVVDADSKIV QLSEISMDNSPNLAWPLIVTALRANSVAKLQ nsp7: MSKMSDVKCTSVVLLSVLQQLRVESSSKLWAQCVQLHNDILLAKDTTEAFEKMSVLLSVLLSMQGAVDINKLCEEMLDNRATLQ				
Handling information: Store at – 80 °C, use immediately after thawing, do not re-freeze.				Released by: KS	

* Measured after one freeze-thaw cycle
† Provided by client

Quality control - nsp12:nsp8:nsp7 PD15376



- Column: S200 increase 10/300
- Running buffer:
10 mM HEPES/NaOH, 150 mM NaCl, 0.5 mM MgCl₂, 10% Glycerole, pH 8.0
- Retention time of globular standard proteins is indicated by dotted lines

- SDS-PAGE shows pure sample (>90% purity); no indication of degradation
 - SDS-PAGE does not show a clear band for nsp7 indicating that this subunits is underrepresented or not present in the sample
- Analytical SEC shows two peaks, presumably corresponding to the individual subunits nsp12 and nsp8
→ Complex is dissociated and the subunits elute individually from aSEC
- Additional peak at the end of the column volume is the result of a slight mismatch between running and storage buffer

[†] Calculated based on concentration provided by client

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