b|e|i RESOURCES
SUPPORTING INFECTIOUS DISEASE RESEARCH

Certificate of Analysis for NR-53713

Spike Glycoprotein (Stabilized) from Human Coronavirus, HKU1 with C-Terminal Histidine and Avi Tags, Recombinant from HEK293F Cells

Catalog No. NR-53713

This reagent is the tangible property of the U.S. Government.

Product Description:

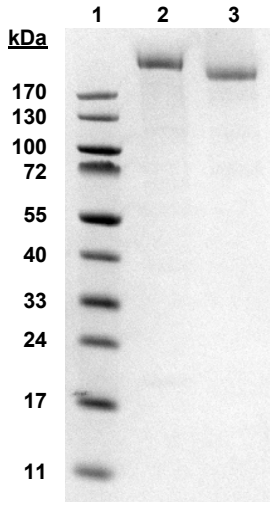
A recombinant form of the spike (S) glycoprotein from human coronavirus (HCoV), HKU1 (GenPept: ABC70719) was produced in human embryonic kidney HEK293F cells and purified by immobilized metal affinity and size exclusion chromatography. NR-53713 lacks the signal sequence and contains 1264 residues (ectodomain) of the HCoV spike glycoprotein; the recombinant protein was stabilized by substitution at the furin S1/S2 cleavage site (RRKRR to GGSGS; residues 752 to 756) and with a pair of mutations (N1067P and L1068P, wild type numbering), and includes a thrombin cleavage site, T4 foldon trimerization domain and C-terminal hexa-histidine tag fused to an AviTag™ BirA biotinylation acceptor sequence.

Lot: 70038217 **Manufacturing Date: 30JUL2020**

TEST	SPECIFICATIONS	RESULTS
Appearance	Clear and colorless	Clear and colorless
Purity		
SDS-PAGE analysis	Protein band of interest represents > 90% of total staining intensity	Protein band of > 170 kDa represents > 90% of total staining intensity (Figure 1)[1]
SEC-HPLC (pre-vial)	Report results	Single peak in elution profile (Figure 2)
Protein Concentration (A_{280})	Report results	1 mg per mL
Final Product		
Amount per vial	Report results	50 µg
Volume per vial	Report results	50 µL
Dynamic Light Scattering	Report results	Aggregate detectable by scattering intensity; negligible aggregate mass (Figure 3)
Filtration	0.22 µm sterile-filtered	0.22 µm sterile-filtered

[1]The recombinant protein migrated to a slightly larger size than was expected, likely caused by glycosylation common in recombinant spike proteins derived from coronaviruses. For more information, please see Chakraborti, S., et al. "The SARS Coronavirus S Glycoprotein Receptor Binding Domain: Fine Mapping and Functional Characterization." Virol. J. 2 (2005): 73. PubMed: 16122388.

Figure 1: SDS-PAGE Analysis



Lane 1: Fisher BioReagents™ EZ-Run™ prestained *Rec* protein ladder
Lane 2: NR-53713 (reduced; 1 µg)
Lane 3: NR-53713 (non-reduced; 1 µg)

Figure 2: SEC-HPLC

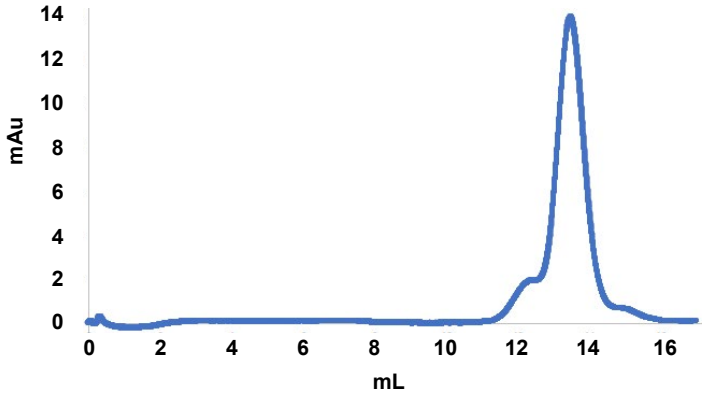


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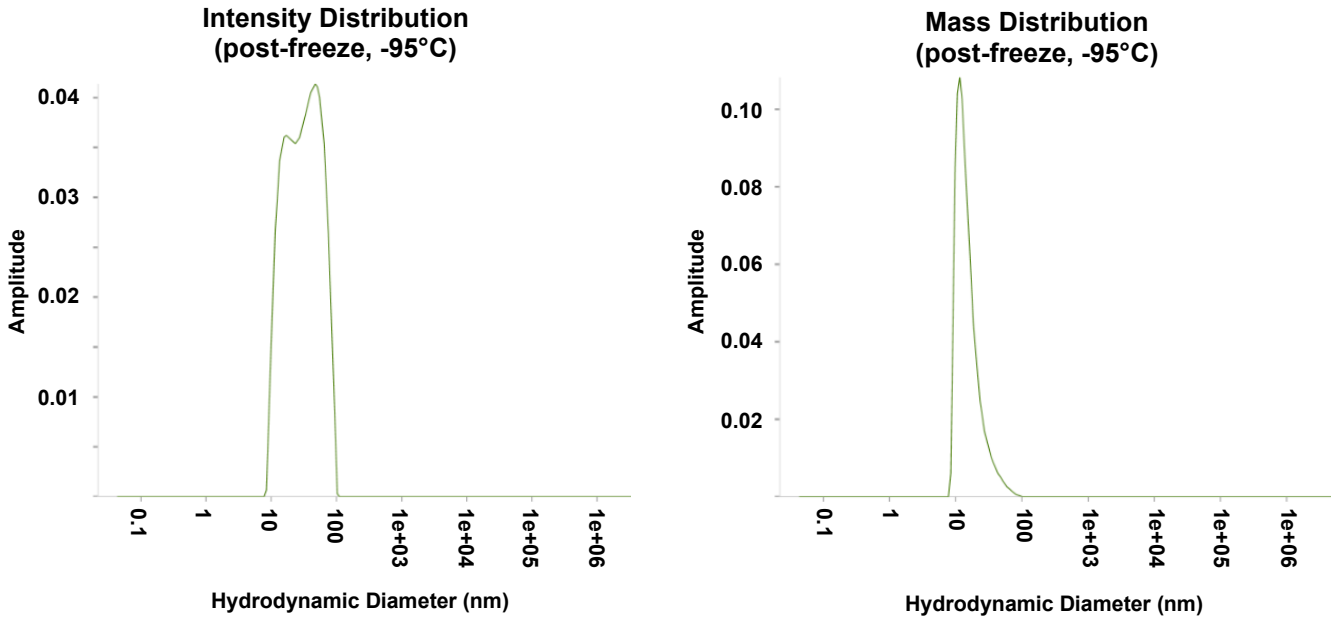
Figure 3: Dynamic Light Scattering Analysis



/Heather Couch/
Heather Couch 17 SEP 2020
Program Manager or designee, ATCC Federal Solutions

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