b|e|i RESOURCES

SUPPORTING INFECTIOUS DISEASE RESEARCH

Certificate of Analysis for NR-53769

Spike Glycoprotein (Stabilized) from SARS-Related Coronavirus 2, Wuhan-Hu-1 HexaPro with C-Terminal Histidine and Twin-Strep® Tags, Recombinant from CHO Cells

Catalog No. NR-53769

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

Product Description:

A recombinant form of the spike (S) glycoprotein from severe acute respiratory syndrome-related coronavirus 2 (SARS-CoV-2), Wuhan-Hu-1 (GenPept: QHD43416) was produced by transfection of purified plasmid (derived from BEI Resources NR-53587) in Chinese hamster ovary (CHO) cells (ExpiCHO-S™; Thermo A29127), purified by immobilized nickel affinity chromatography (cOmplete™ His-Tag Resin) and vialed in 20 mM Tris, pH 8.0 and 500 mM NaCl. NR-53769 lacks the signal sequence and contains 1194 residues (ectodomain) of the SARS-CoV-2 S glycoprotein; the recombinant protein was stabilized by substitution at the furin S1/S2 cleavage site (RRAR→GSAS; residues 682 to 685) and KV→PP mutations (residues 986 and 987) as well as the additional proline substitutions that create the HexaPro variant (F817P, A892P, A899P and A942P), and includes a T4 foldon trimerization domain, HRV3C protease cleavage site, and C-terminal octa-histidine and Twin-Strep® (TST) tags.

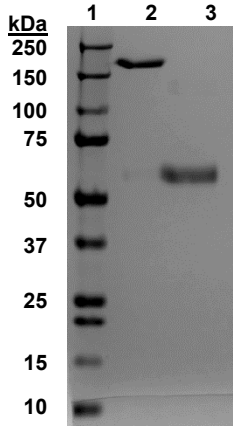
Lot: 70040974 **Manufacturing Date: 06JAN2021**

TEST	SPECIFICATIONS	RESULTS
Appearance	Clear and colorless	Clear and colorless
SDS-PAGE Analysis (Coomassie Blue)	Protein band of interest represents > 90% of total staining intensity	Protein band of approximately 190 kDa represents 100% of total staining intensity (Figure 1)[1]
Concentration by Bicinchoninic Acid Assay		
Bovine Serum Albumin (standard)	Report results	0.56 mg per mL
Final Product		
Amount per vial	Report results	45 µg
Volume per vial	Report results	80 µL
Functional Activity by Western Blot Analysis		
Monoclonal anti-histidine tag	Reactive	Reactive (Figure 2)[2]
Filtration	0.2 µm sterile-filtered	0.2 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.

[2]Using a 1:1000 dilution of mouse monoclonal anti-histidine tag (R&D Systems MAB050) as primary antibody and a 1:1000 dilution of HRP-conjugated goat anti-mouse IgG (R&D Systems HAF007) as secondary antibody.

Figure 1: SDS-PAGE Analysis



Lane 1: Precision Plus Protein™ Standard (5 µL)
Lane 2: NR-53769 (1 µg)
Lane 3: Bovine serum albumin (1 µg)

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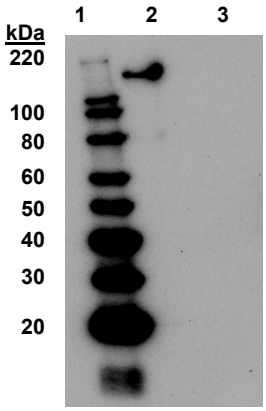
Figure 2: Anti-Histidine Western Blot Analysis



Lane 1: MagicMark™ XP Protein Standard (5 µL)
Lane 2: NR-53769 (0.2 µg)
Lane 3: Bovine serum albumin (0.2 µg)

/Heather Couch/
Heather Couch 14 APR 2021
Program Manager or designee, ATCC Federal Solutions

ATCC®, on behalf of BEI Resources, hereby represents and warrants that the material provided under this certificate has been subjected by ATCC® to the tests and procedures specified and that the results described, along with any other data provided in this certificate, are true and accurate to the best of ATCC®'s knowledge.

Support Provided by NIAID

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