b|e|i RESOURCES

SUPPORTING INFECTIOUS DISEASE RESEARCH

Certificate of Analysis for NR-52397

Spike Glycoprotein (Stabilized) from SARS-Related Coronavirus 2, Wuhan-Hu-1 with C-Terminal Histidine Tag, Recombinant from HEK293F Cells

Catalog No. NR-52397

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: QJE37812) was produced by transfection of purified plasmid (derived from BEI Resources NR-52394) in human embryonic kidney HEK293F (Expi293F™; Gibco™ A14527) cells, purified by nickel affinity (Ni-NTA agarose) chromatography and vialed in phosphate-buffered saline (PBS), pH 7.4. NR-52397 lacks the signal sequence and contains 1196 residues (ectodomain) of the SARS-CoV-2 spike glycoprotein; the recombinant protein was modified to remove the polybasic S1/S2 cleavage site (RRAR to A; residues 682 to 685), stabilized with a pair of mutations (K986P and V987P, wild type numbering; GenPept: YP_009724390) and includes a thrombin cleavage site, T4 foldon trimerization domain and C-terminal hexa-histidine tag.

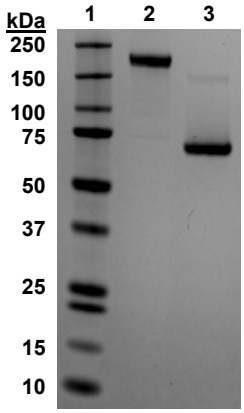
Lot: 70040292 **Manufacturing Date: 08JAN2021**

TEST	SPECIFICATIONS	RESULTS
Appearance	Clear and colorless	Clear and colorless
SDS-PAGE Analysis	Protein band of interest represents > 90% of total staining intensity	Protein band of approximately 200 kDa represents > 90% of total staining intensity (Figure 1)[1]
Concentration by Bicinchoninic Acid Assay Bovine Serum Albumin (standard)	Report results	0.146 mg per mL
Vial Contents Amount per vial Volume per vial	 Report results Report results	 0.026 mg 0.175 mL
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.

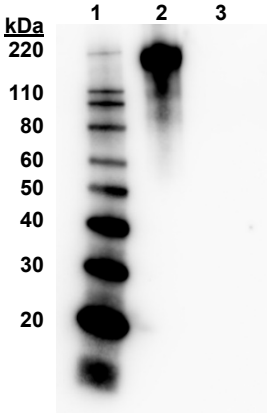
[2]Using a 1:1000 dilution of mouse monoclonal anti-histidine tag (R&D Systems MAB050) as primary antibody and a 1:1000 dilution of HRP-conjugated goat anti-mouse IgG (R&D Systems HAF007) as secondary antibody.

Figure 1: SDS-PAGE Analysis



Lane 1: Precision Plus Protein™ Standard (4 µL)
Lane 2: NR-52397 (1 µg)
Lane 3: Bovine serum albumin (1 µg)

Figure 2: Anti-Histidine Western Blot Analysis



Lane 1: MagicMark™ XP Protein Standard (6 µL)
Lane 2: NR-52397 (0.2 µg)
Lane 3: Bovine serum albumin (0.2 µg)

BEI Resources
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/Heather Couch/
Heather Couch 02 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 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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