

DESCRIPTION

Source	Human embryonic kidney cell, HEK293-derived sars-cov-2 Spike protein		
	<p>SARS-CoV-2 P.1 Spike (Val16-Lys1211)(Leu18Phe, Thr20Asn, Pro26Ser, Asp138Tyr, Arg190Ser, Lys417Thr, Glu484Lys, Asn501Tyr, Asp614Gly, His655Tyr, Thr1027Iso, Val1176Phe) (Arg682Ser, Arg685Ser, Lys986Pro, Val987Pro) Accession # YP_009724390.1</p>	GCN4-IZ	6-His tag
	N-terminus		C-terminus
N-terminal Sequence Analysis	Val16		
Structure / Form	Labeled with Alexa Fluor® 647		
	Excitation Wavelength: 650 nm Emission Wavelength: 668 nm		
Predicted Molecular Mass	138 kDa		

SPECIFICATIONS

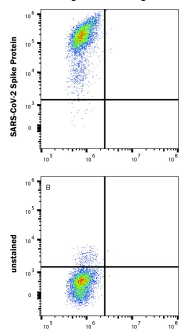
SDS-PAGE	140-170 kDa, under reducing conditions.
Activity	Measured by flow cytometry for its ability to bind HEK293 human embryonic kidney cells transfected with human ACE-2 at 0.25-1.00 µg/mL (100 µL/well). Please Note: Optimal dilutions should be determined by each laboratory for each application.
Endotoxin Level	<1.0 EU per 1 µg of the protein by the LAL method.
Purity	>95%, by SDS-PAGE visualized with Silver Staining and quantitative densitometry by Coomassie® Blue Staining.
Formulation	Supplied as a 0.2 µm filtered solution in PBS with BSA as a carrier protein. See Certificate of Analysis for details.

PREPARATION AND STORAGE

Shipping	The product is shipped with dry ice or equivalent. Upon receipt, store it immediately at the temperature recommended below.
Stability & Storage	<p>Protect from light. Use a manual defrost freezer and avoid repeated freeze-thaw cycles.</p> <ul style="list-style-type: none"> • 6 months from date of receipt, -20 to -70 °C as supplied. • 1 month, 2 to 8 °C under sterile conditions after opening. • 3 months, -20 to -70 °C under sterile conditions after opening.

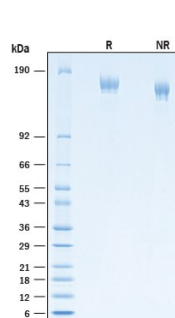
DATA

Flow Cytometry



Detection of ACE-2 on HEK293 Transfectants with Recombinant SARS-CoV-2 P.1 Spike (GCN4-IZ) His-tag Alexa Fluor® 647 by Flow Cytometry. HEK293 human embryonic kidney cells transfected with human ACE-2 were stained with (A) 1 µg/mL (100 µL/well) Recombinant SARS-CoV-2 P.1 Spike (GCN4-IZ) His-tag Alexa Fluor® 647 Protein (Catalog # AFR10795) or (B) unstained.

SDS-PAGE



Recombinant SARS-CoV-2 P.1 Spike (GCN4-IZ) His-tag Alexa Fluor® 647 Protein SDS-PAGE. 2 µg/lane of Recombinant SARS-CoV-2 P.1 Spike (GCN4-IZ) His-tag Alexa Fluor® 647 Protein (Catalog # AFR10795) was resolved with SDS-PAGE under reducing (R) and non-reducing (NR) conditions and visualized by Coomassie® Blue staining, showing bands at 140-170 kDa.

BACKGROUND

SARS-CoV-2, which causes the global pandemic coronavirus disease 2019 (Covid-19), belongs to a family of viruses known as coronaviruses that also include MERS-CoV and SARS-CoV-1. Coronaviruses are commonly comprised of four structural proteins: Spike protein (S), Envelope protein (E), Membrane protein (M) and Nucleocapsid protein (N) (1). The SARS-CoV-2 S protein is a glycoprotein that mediates membrane fusion and viral entry. The S protein is homotrimeric, with each ~180-kDa monomer consisting of two subunits, S1 and S2 (2). In SARS-CoV-2, as with most coronaviruses, proteolytic cleavage of the S protein into S1 and S2 subunits is required for activation. The S1 subunit is focused on attachment of the protein to the host receptor while the S2 subunit is involved with cell fusion (3-5). The S protein of SARS-CoV-2 shares 75% and 29% amino acid sequence identity with S protein of SARS-CoV-1 and MERS, respectively. The S Protein of the SARS-CoV-2 virus, like the SARS-CoV-1 counterpart, binds a metalloproteinase, Angiotensin-Converting Enzyme 2 (ACE-2), but with much higher affinity and faster binding kinetics through the receptor binding domain (RBD) located in the C-terminal region of S1 subunit (6). It has been demonstrated that the S Protein can invade host cells through the CD147/EMMPRIN receptor and mediate membrane fusion (7, 8). Polyclonal antibodies to the RBD of the SARS-CoV-2 protein have been shown to inhibit interaction with the ACE-2 receptor, confirming RBD as an attractive target for vaccinations or antiviral therapy (9). There is also promising work showing that the RBD may be used to detect presence of neutralizing antibodies present in a patient's bloodstream, consistent with developed immunity after exposure to the SARS-CoV-2 (10). Several emerging SARS-CoV-2 genomes have been identified including the P.1 (Brazilian) variant (11). The P.1 variant contains 3 mutations of interest in the RBD domain: K417T, E484K, and N501Y (11). The E484K substitution alone has been shown to confer resistance to several monoclonal antibodies and is responsible for the first confirmed SARS-CoV-2 reinfection (12). Structural analysis points to E484K as a potentially crucial mutation as it creates a new site for hACE-2 binding and may enhance binding affinity (13).

References:

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