Miltenyi Biotec

Recombinant SARS-CoV-2 Spike-Prot B.1.351 (HEK)

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1. Description

Products Recombinant SARS-CoV-2 Spike-Prot B.1.351 (HEK) or Recombinant SARS-CoV-2 Spike-Prot B.1.351 (HEK)-Biotin.

Productname	Content	Order no.
Recombinant SARS-CoV-2 Spike-Prot B.1.351 (HEK)	10 µg	130-129-559
Recombinant SARS-CoV-2 Spike-Prot B.1.351 (HEK)	50 µg	130-129-560
Recombinant SARS-CoV-2 Spike-Prot B.1.351 (HEK)-Biotin	25 µg	130-129-569

Functional testing Functionality was tested in a flow assay by analyzing the binding of Recombinant SARS-CoV-2 Spike-Prot B.1.351 to ACE2-expressing cells.

Primary structure Recombinant SARS-CoV-2 Spike-Prot B.1.351 (HEK): Single glycosylated polypeptide chain (1230 amino acid residues) without N-terminal methionine. C-terminal His-tag and C-terminal AviTag™.

Recombinant SARS-CoV-2 Spike-Prot B.1.351 (HEK)-Biotin: Monobiotinylated, glycosylated polypeptide chain (1230 amino acid residues) without N-terminal methionine. C-terminal His-tag and C-terminal AviTag

Molecular mass 135.8 kDa.

Source Produced in human embryonic kidney cells.

Product format Lyophilized from a filtered (0.2 µm) buffer solution.

Stabilizer Mannitol and trehalose.

Purity >95% as determined by SDS-PAGE analysis.

Endotoxin level Low endotoxin (<0.25 EU/µg protein) as determined by Limulus Amebocyte Lysate (LAL) assay.

Storage Lyophilized Recombinant SARS-CoV-2 Spike Prot B.1.351 should be stored at –20 °C. The expiration date is indicated on the vial label. Upon reconstitution aliquots should be stored at –20 °C or below. Avoid repeated freeze-thaw cycles.

Reconstitution It is recommended to reconstitute lyophilized Recombinant SARS-CoV-2 Spike Prot B.1.351 with deionized, sterile-filtered water to a final concentration of 0.02–0.25 mg/mL in a minimal volume of 200 µL.

1.1 Background information

The SARS-CoV-2 spike glycoprotein (S) mediates the entry of the virus into target cells via its interaction with the angiotensin-converting enzyme 2 (ACE2) receptor, thereby initiating the infection. It forms a homotrimeric structure on the surface of the SARS-CoV-2 virus and represents the major target for diagnostic and therapeutic agents. The ectodomain of the S glycoprotein comprises the N-terminal S1 subunit, including the receptor binding domain (RBD) and the C-terminal S2 subunit. The ectodomain spans from aa12 to aa1213 followed by a transmembrane helix beginning at aa1214–aa1234. The C-terminus of the native protein is formed by a cytoplasmic domain spanning aa1235–aa1273.

The SARS-CoV-2 mutant strain B.1.351, also known as 501Y.V2 or variant Beta, was first identified in of South Africa in December 2020. Due to increased transmissibility it spread rapidly around the globe. The B.1.351 lineage (Beta variant) differs from the wild-type sequence by a total of 21 amino acid mutations of which three key amino acid substitutions K417N, E484K, and N501Y occur in the RBD. The N501Y mutation also emerged independently in SARS-CoV-2 variants of the B.1.1.7 lineage (Alpha variant) and P.1 lineage (Gamma variant). The B.1.351 lineage (Beta variant) is reported to have a higher infectivity due to the increased affinity to the ACE2 receptor and shows reduced susceptibility to neutralizing antibodies.

The Recombinant SARS-CoV-2 Spike-Prot B.1.351 (HEK) protein covers the ectodomain of the viral surface protein including amino acids V16 to K1211. It was engineered to contain the stabilizing proline substitutions at position K986P and V987P. The native sequence of the Furin cleavage site (RRAR at residues 682-685) was substituted by GSAS to further increase recombinant protein stability.

In addition, the SARS-CoV-2 Spike-Prot B.1.351 (HEK) protein contains the amino acid substitutions L18F, D80A, D215G, R246I, K417N, E484K, N501Y, D614G, and A701V. The protein is extended at its C-terminus with a His-tag and an AviTag. Recombinant SARS-CoV-2 Spike-Prot B.1.351 (HEK)-Biotin is specifically biotinylated at a single site, preserving full functionality of the protein.

1.2 Applications

The product can be used for a variety of applications, including:

- Analysis of SARS-CoV-2 immune responses
- Detection and characterization of SARS-CoV-2–specific antibodies
- Studying SARS-CoV-2 cell binding, infection, and viral life cycle

The optimal concentration for a specific application should be determined by a dose-response experiment.

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