

F (SEQ ID NO: 14)

[0197] A side-by-side comparison of the SARS-CoV-2 native SP/RBD (SEQ ID NO: 2) and the SP/RBD fragment (SEQ ID NO: 14) was performed using Clustal Omega and is shown in FIG. 15. “*” represents complete homology across all sequences at a given sequence position. A “:” (colon) indicates conservation between groups of strongly similar properties with scoring greater than 0.5 in the Gonnet PAM 250 matrix. A “-” (dash) indicates a sequence gap, meaning that no local homology exists within a particular set of comparisons within a certain range of the sequences, while “:”, “.” or spaces refer to conservative, moderate, or very different amino acid mutations across the sequences at a given sequence position respectively.

[0198] In one attempt, the linker was also shortened and changed in composition to GGGSGGG (SEQ ID NO: 3), and the hinge region and the C-terminal lysine on the human IgG1 Fc fragment was restored, producing the Fc fragment of SEQ ID NO: 7.

SPKSSDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPE
VKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVE
WESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFS CSVMHEALHN
HYTQKSLSLSPGK (SEQ ID NO: 7)

[0199] The asparagine at the cNg site on the human IgG1 fragment was conserved to preserve the glycan attachment during fusion protein production in host cells. The resulting SARS-CoV-2-RBD-hIgG-Fc fusion protein (SEQ ID NO: 17) is shown below.

RVQPTESIVRFPNITNLCPFGEVFNATRFASVYAWNRKRISNCVADYSVLVNSASF
STFKCYGVSPTKLNDLCFTNVYADSFVIRGDEVQRQIAPGQTGKIADYNYKLPPDDF
TGCVIAWNSNNLDSKVGGNYNYLYRLFRKSNLKPFERDISTEIQAGSTPCNGVE
GFNCYFPLQSYGFQPTNGVGYQPYRVVLSFELLHAPATVCGPKKSTNLVKNKC
VNFGGGSGGGSPKSSDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTC
VVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDW
LNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLV
KGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFS
CSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 17)

[0200] The Fc(gamma) Receptor I binding of the SARS-CoV-2-RBD-hIgG-Fc fusion protein of SEQ ID NO: 17 manufactured in HEK293 cells according to Example 1 is measured according to Example 9. It is expected that the OD450 measurements for Fc(gamma) Receptor I, Fc(gamma) Receptor IIA, Fc(gamma) Receptor IIB, Fc(gamma) Receptor III, FcRn, and ACE2 receptor binding will increase as a function of the concentration of the SARS-CoV-2-RBD-hIgG-Fc fusion protein.

[0201] In a further attempt, the SARS-CoV-2 RBD fragment of SEQ ID NO: 14 and the linker of SEQ ID NO: 3 were retained, and the C-terminal lysine on the human IgG1 Fc fragment was removed again, producing the Fc fragment of SEQ ID NO: 33.

SPKSSDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPE
VKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSN
KALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVE
WESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSQSVSMHEALHN
HYTQKSLSLSPG (SEQ ID NO: 33)

[0202] The asparagine at the cNg site on the human IgG1 fragment was conserved to preserve the glycan attachment during fusion protein production in host cells. The resulting SARS-CoV-2-RBD-hIgG-Fc fusion protein is shown below.

RVQPTESIVRFPNITNLCPFGEVFNATRFASVYAWNRKRISNCVADYSVLVNSASF
STFKCYGVSPTKLNDLCFTNVYADSFVIRGDEVQRQIAPGQTGKIADYNYKLPDDF
TGCVIAWNSNNLDSKVGGNYNYLYRLFRKSNLKPFERDISTEIQAGSTPCNGVE
GFNCYFPLQSYGFQPTNGVGYQPYRVVLSFELLHAPATVCGPKKSTNLVKNKC
VNFGGGSGGGSPKSSDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTC
VVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDW
LNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLV
KGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFS
CSVMHEALHNHYTQKSLSLSPG (SEQ ID NO: 16)

[0203] The Fc fusion protein of SEQ ID NO: 16 was recombinantly manufactured in transiently transfected CHO cells according to Example 2. The Fc fusion protein of SEQ ID NO: 16 was purified according to Example 5. The fusion protein structure of SEQ ID NO: 16 was confirmed according to Example 6, and the sequence identification was performed according to Example 7. To obtain the homodimer titer of the manufactured SARS-CoV-2-RBD-hIgG-Fc fusion protein, the %homodimer was measured according to Example 8 and the homodimer titer was calculated by multiplying the %homodimer by the protein titer of the recombinantly manufactured fusion protein. The homodimer titer of the SARS-CoV-2-RBD-hIgG-Fc fusion protein of SEQ ID NO: 16 was calculated to be 139 mg/L.

[0204] The Fc(gamma) Receptor I binding of the SARS-CoV-2-RBD-hIgG-Fc fusion protein of SEQ ID NO: 16 manufactured in transiently transfected CHO cells according to Example 2, or in CHO cells according to Example 3 is measured according to Example 9. It is expected that the OD450 measurements for Fc(gamma) Receptor I, Fc(gamma) Receptor IIA, Fc(gamma) Receptor IIB, Fc(gamma) Receptor III, FcRn, and ACE2 receptor binding will increase as a function of the concentration of the SARS-CoV-2-RBD-hIgG-Fc fusion protein.