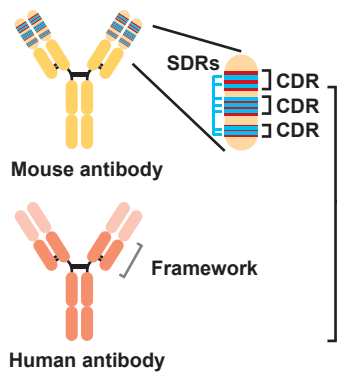


Custom Service for CDR Grafting

Abnova's humanized antibody service starts with mouse monoclonal antibody production via mouse immunization, splenic fusion, hybridoma screening, antibody selection, and isotype determination. The selected mouse monoclonal antibody undergoes variable gene sequencing for complementarity-determining region (CDR) grafting, and if needed specificity determining residues (SDR) grafting, onto the human antibody construct followed by sequence alignment and production. The humanized antibody is then characterized by a series of in vitro and in vivo assays to assure its biochemical, biophysical, and functional properties. Moreover, the Fc region of the humanized antibody is mutated to minimize antibody-dependent enhancement (ADE) immune response.

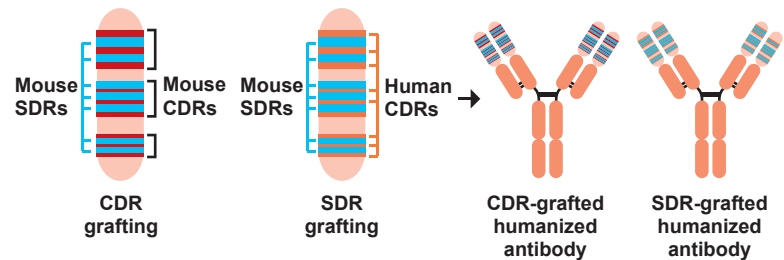
Monoclonal Antibody Production

- Antigen Preparation
- Mouse Immunization
- Hybridoma Screening
- Antibody Selection
- Isotype Determination



Monoclonal Antibody Humanization

- Mouse Antibody Variable Gene Sequencing
- Complementarity-Determining Region (CDR) Grafting
- Specificity Determining Residue (SDR) Grafting
- Humanized Antibody Sequence Alignment
- Humanized Antibody Variant Production

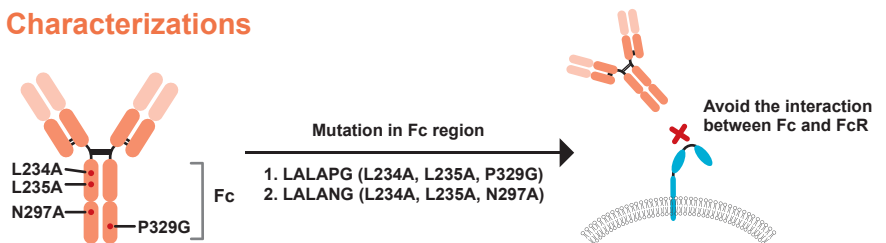


Humanized Antibody Characterizations

- Protein and Peptide Mapping
- Affinity Kinetic Assay
- Binding, Inhibition, Neutralization Assays
- Cytokine, ADCC/CDC, and Toxicology Assays
- Animal Assay

Humanized Antibody Characterizations

- LALAPG mutation
- LALANG mutation



Example

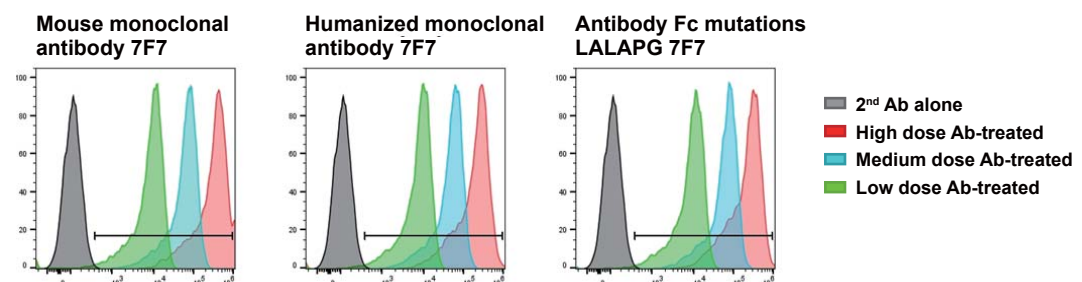
COVID-19 Humanized Monoclonal Antibody (clone 7F7)

■ Antibody Binding Specificity

- 293T-SARS-CoV-2 spike cells
- Mouse monoclonal antibody 7F7
- Humanized monoclonal antibody 7F7
- Antibody Fc mutation LALAPG 7F7
- 293T-SARS-CoV-2 spike cells were treated with mouse 7F7, humanized 7F7, and LALAPG 7F7 antibody. Data were analyzed by flow cytometer.

Data

- Mouse 7F7, humanized 7F7, and LALAPG 7F7 all efficiently bound to 293T-SARS-CoV-2 spike cells.

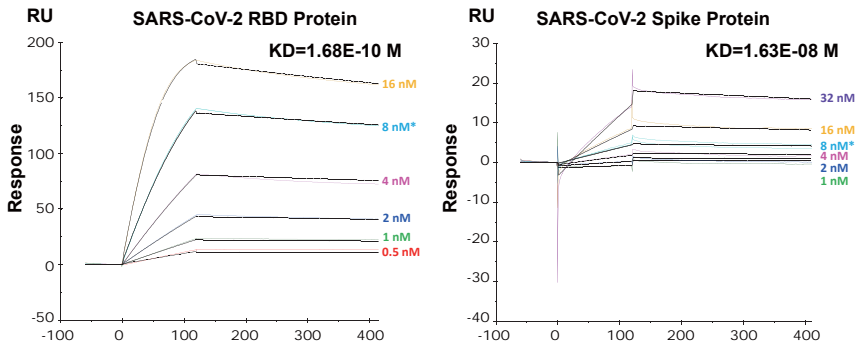


Antibody Kinetic Assay

Biacore Surface Plasmon Resonance

Data

- Mouse Monoclonal Antibody 7F7:

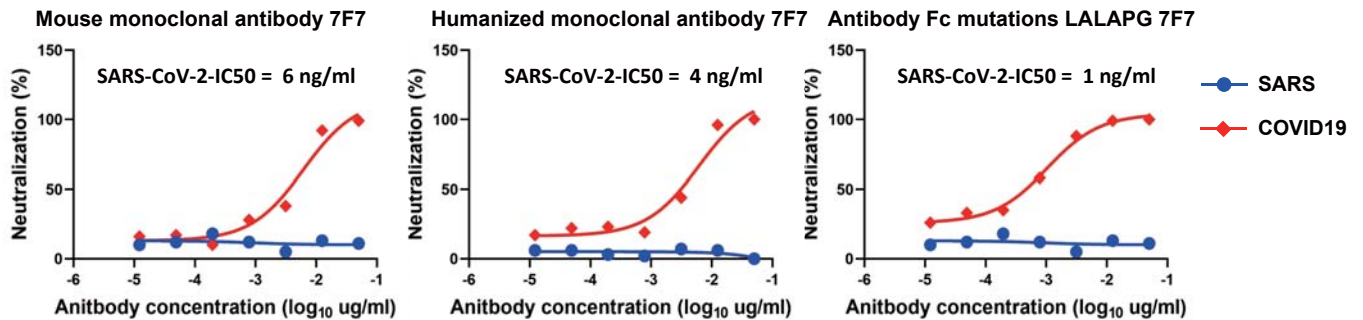


COVID-19 Antibody	SARS-CoV-2 RBD Protein	SARS-CoV-2 Spike Protein
	KD (M)	KD (M)
Mouse Monoclonal Antibody 7F7	1.68E-10	1.63E-08
Humanized monoclonal antibody 7F7	1.34E-10	1.87E-10
Antibody Fc mutations LALAPG 7F7	7.13E-11	3.85E-10

Pseudovirus Neutralization Assay

- 293T-ACE2 cells
- SARS-CoV-2 spike pseudovirus expressing luciferase
- Pseudovirus was treated with mouse 7F7, humanized 7F7, and LALAPG 7F7 antibody, and then added into 293T-ACE2 cells. Luciferase activity was measured 48 hours post infection.

Data



CD3 Humanized Monoclonal Antibody

Antibody Binding Specificity

- Human CD3 T cells
- Mouse monoclonal antibody
- Humanized CD3 monoclonal antibody
- Antibody Fc mutation LALAPG
- Human CD3 T cells were treated with mouse, humanized, and LALAPG antibody. Data were analyzed by flow cytometer.

Data

- Mouse, humanized, and LALAPG antibodies all efficiently bound to CD3 T cells.

