# Identification and Characterization of Pan-Variant RBD-Specific Antibodies

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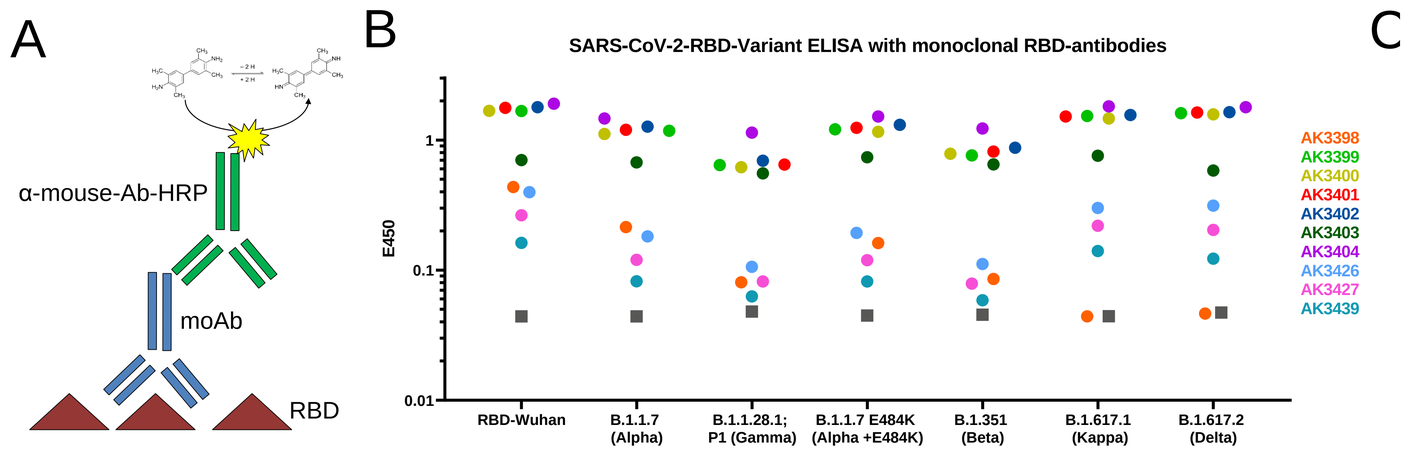
## Abstract

Currently used workflows are often focused on a single target protein. This may limit the application of antibodies for diagnostics and drug development especially for fast mutating proteins like the Spike protein of severe acute respiratory syndrome coronavirus type 2 (SARS-CoV-2). We report a workflow aiming to develop and characterize antibodies against the receptor binding domain (RBD) of the SARS-CoV-2 Spike Protein with pan-variant activity. Mice were immunized with a recombinant form of the RBD (Wuhan-Hu-1-isolate, MN908947, here termed: wild type) produced in HEK-INV cells. Harvested, monoclonal antibodies were tested for their affinity towards different RBD variants of concern by ELISA. They were further characterized concerning their ability to inhibit the binding of the Spike protein to Angiotensin-converting enzyme 2 (ACE2), a transmembrane protein located on various mammalian cells, which serves as an entry point for SARS-CoV-2. The four best performing antibodies also showed activity in a neutralization assay for the Wuhan wild type, the alpha (B.1.1.7) and beta (B.1.351) variant. A subpanel was additionally characterized for their binding kinetics against RBDs from well documented variants by SPR with novel throughput optimized assay format on the Sierra SPR-32 Pro system. Generally, a reduction in affinity and the kinetic rate constants was observed which aided in identifying antibodies with consistent, panel-wide binding kinetics.

## Discovery of Specific and Efficient Antibodies




| | $EC_{50}$ WT [µg/ml] | $EC_{50}$ UK [µg/ml] | $EC_{50}$ SA [µg/ml] |
|---|---|---|---|
| **AK3398** | 0.004216 | 0.003616 | 0.004933 |
| **AK3400** | 0.3006 | 0.1366 | 0.1197 |
| **AK3402** | 0.1624 | 0.1186 | 0.1599 |
| **AK3404** | 0.1665 | 0.2293 | 0.3165 |

Figure 1: A: Principle of ELISA-assay for affinity analysis. RBD-variants were immobilized and bound antibodies detected with a secondary antibody. B: ELISA-Screen of tested antibodies against RBD-variants. C: Competition assay of selected antibodies blocking the binding of SARS-CoV-2 Spike protein to ACE2. D: In-cell neutralization assay performed with Vero6 cells for best performing antibodies in competition assay. $EC_{50}$ are listed in the table. Best performing with all three virus variants is AK3398. Neutralization assays were performed by our cooperation partner Dr. Valeria Falcone from the Institute of Virology at the University Clinic Freiburg.

## The Titration Cycle Kinetics Assay Format

The more efficient SPR-assay format TCK is a multi-association/single dissociation format that helped to identify four antibodies with an overall higher binding capacity for all RBD-variants. Mutations in the RBD affected both the association as well as dissociation rate constant for all antibodies, yet the dissociation rate constant was stronger affected.

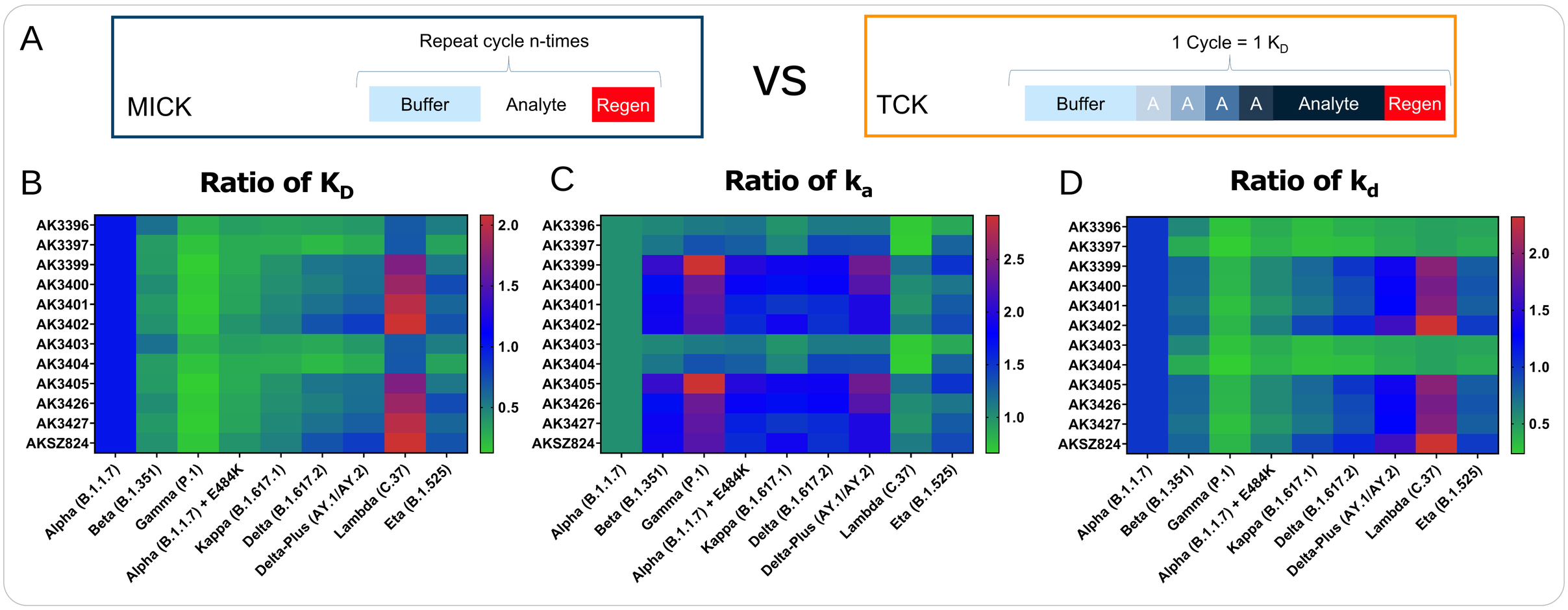



Figure 2: A: Schematic illustration of MICK (left) and TCK (right) assay format. B-D: Heatmaps of the ratio for affinity $K_D$ (B), association rate constant $k_a$ (C) and dissociation rate constant $k_d$ (D) of the RBD-variant screen against 12 antibodies.

## Acknowledgements

We thank Dr. Valeria Falcone from the Institute of Virology at the University Clinic Freiburg for performing the neutralization assay.

Surface Plasmon Resonance