



## Special Instructions for ACE2 Assays with Omicron Antigens

MSD's ACE2 Neutralization Assays are usually supplied with a monoclonal antibody that functions as a calibration reagent. The monoclonal antibody present in the ACE2 Calibrator Reagent (Catalog No. C01ADG-2) does not bind to the Omicron Spike and RBD. MSD now ships a new ACE2 Calibrator Reagent 2 (Catalog No. C01AJZ-2) for assay kits containing Omicron Spike or RBD. The ACE2 Calibration Reagent 2 includes a blend of monoclonal antibodies that bind to all SARS-CoV-2 variants, except Omicron sublineages BA.2.75.2, BA.4, BA.4.6, BA.5, BF.7, BQ.1, BQ.1.1, XBB.1, and XBB.1.5.

- **Recommended Protocol:** Users should follow the recommended protocol outlined in the V-PLEX® COVID-19 ACE2 Neutralization Assays Insert.
- **Analysis of Results:** Analysis follows guidance in Step 5 of the recommended protocol in the V-PLEX COVID-19 ACE2 Neutralization Assays Insert. Results for BA.2.75.2, BA.4, BA.4.6, BA.5, BF.7, BQ.1, BQ.1.1, XBB.1, and XBB.1.5 can be reported as percent inhibition, calculated using the equation below. Highly positive samples show a high percentage of inhibition, whereas negative or samples with low concentrations show low percent inhibition.

$$\% \text{ Inhibition} = 1 - \frac{\text{Average Sample ECL Signal}}{\text{Average ECL signal of Calibrator 8 (Diluent 100 only)}} \times 100$$

Calculating neutralizing antibody concentrations for Omicron sublineages BA.2.75.2, BA.4, BA.4.6, BA.5, BF.7, BQ.1, BQ.1.1, XBB.1, and XBB.1.5 by backfitting the measured ECL signals for samples to a calibration curve is not currently supported.

- **ECL Signals and Sample Dilution:** ECL signals for ACE2 measurements on the Omicron Spike and RBD spots are lower than signals measured with other SARS-CoV-2 variants but still sufficient to achieve good results. We don't recommend trying to increase ECL signals for Omicron by increasing the concentration of the labeled ACE2 reagent. Increasing the concentration of the labeled ACE2 reagent will create unusually high ECL signals on the spots for other SARS-CoV-2 variants, possibly compromising the results of the assays. We advise testing at the recommended sample dilutions (10-fold to 100-fold dilution in Diluent 100) provided in the "Sample Preparation" section of the V-PLEX COVID-19 ACE2 Neutralization Assays Insert unless the user empirically determines that other dilutions work better.

If you need further assistance, please contact the MSD Scientific Support team at +1 240 314 2798 or [scientificsupport@mesoscale.com](mailto:scientificsupport@mesoscale.com).