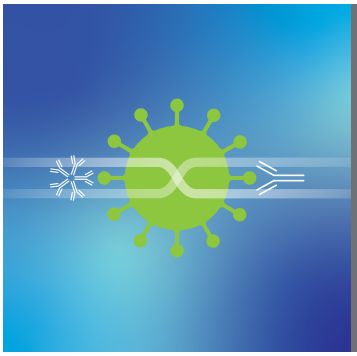

Bio-Plex™ Pro Human SARS-CoV-2 Neutralization Antibody Assays

High Performance Multiplex SARS-CoV-2 Neutralization Antibody Assays against SARS-CoV-2 Wild-Type and Variant Antigens

MAGNETIC SEPARATION ENABLED

Neutralizing antibodies against 13 SARS-CoV-2 antigens:

- Receptor binding domain (RBD)
- Spike 1 (S1)
- Alpha S1
- Beta S1
- Gamma RBD
- Delta RBD
- Delta Spike Trimer
- Epsilon RBD
- Kappa RBD
- D614G S1
- E484K RBD
- K417N RBD
- N501Y RBD

- Fast time to results
- High precision and specificity
- Flexible assay configurations

Neutralizing antibodies to severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) prevent virus entry into cells' angiotensin converting enzyme 2 (ACE2) receptors by binding to part of the viral spike protein, preventing infection. The spike protein subunits S1 and RBD are prime targets for neutralizing antibodies because of their role in viral entry into host cells via the ACE2 receptor. Mutations in these regions can result in SARS-CoV-2 variants that are more infectious and transmissible.

Bio-Plex Pro Human SARS-CoV-2 Neutralization Antibody Assays provide researchers assessing the efficacy of coronavirus disease 2019 (COVID-19) vaccines, or comparing the effectiveness of naturally acquired and vaccine-induced antibody response to arising variants of interest or concern, highly precise assays to quantitatively measure SARS-CoV-2 neutralizing antibodies against 2 wild-type and 11 variants of S1 and RBD protein subunits. SARS-CoV-2 antigens are coated on magnetic beads that bind neutralizing antibodies in competition with an ACE2 receptor that has been biotinylated for detection.

There is a need to understand the longevity of the humoral immune response and how effective neutralizing antibodies are against arising variants of interest or concern. Assessing the protection provided by antibodies (including monoclonal therapies as well as neutralizing antibodies generated by the immune system after natural infection or vaccination) against newly arising variants is essential for the protection of individuals and communities. The outcome of these assessments can enable public health researchers and therapeutic developers to attribute appropriate levels of concern to arising variants.

These research use only assays provide a fast and efficient way to simultaneously measure neutralizing antibodies against 2 wild-type and 11 variants of SARS-CoV-2 in <3 hours. In comparison, traditional cell-based methods and enzyme-linked immunosorbent assays (ELISAs), are lower throughput and require multiple days to get the same information.

For quantitative assays, a standard and a positive control are included in the kit. The competitive assay format results in a standard curve where the signal is inversely proportional

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to the concentration of neutralizing antibodies in the sample. This results in lower median fluorescence intensity (MFI) for samples that have higher levels of neutralizing antibodies. Sample interpretation guidelines provide concentration cutoff values for quantitative assays, which indicate whether samples are positive for neutralizing antibodies to the SARS-CoV-2 antigen. The percentage inhibition of the neutralizing antibodies in samples can be compared by measuring the MFI of the sample versus that of the negative control, which is a well that receives coupled beads, serology sample diluent, biotyinylated detection ACE2 receptor, and streptavidin-phycoerythrin (SA-PE). The calculation for percentage inhibition is:

Percentage inhibition = 1 – (MFI of sample/MFI of negative control) x 100

The Bio-Plex Pro SARS-CoV-2 Delta RBD and Delta Spike Trimer Assays are qualitative. For qualitative assays, percentage inhibition cutoff values are provided. If samples are equal to or above the percentage inhibition cutoff they are positive for neutralizing antibodies against the SARS-CoV-2 antigen.

Assay Features

- Reduced turnaround time
- Reproducible results
- Specific for SARS-CoV-2 wild-type and variant S1 and RBD
- Flexible assay configurations
- Antigen-coupled beads and reagents for multispecies studies

Rigorous Assay Validation

- Specificity (cross-reactivity)
- Inter-and intra-assay precision
- Correlation with COVID-19 and serum and plasma samples from healthy subjects

Bio-Plex Pro Human SARS-CoV-2 Neutralization Antibody Assay Characteristics

Representative Assay and Performance Characteristics

Parameter	Characteristic
Reactive species	Human
Analytical specificity (analyte cross-reactivity)	<5
Intra-assay precision, %CV	<10
Inter-assay precision, %CV	<20
Compatible sample matrices	Serum, plasma

%CV, coefficient of variation.

Clinical Specificity and Sensitivity Results

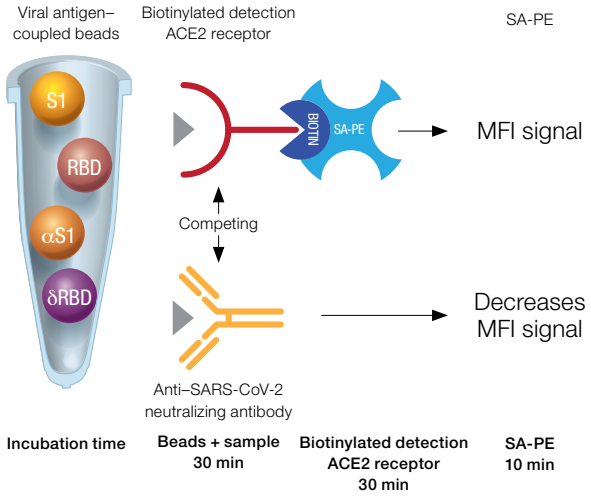
SARS-CoV-2 Wild-Type and Variant Antigens	Clinical Specificity, % n = 118, Delta n = 114*	Clinical Sensitivity, % n = 84, Delta n = 76**
RBD	100	98
S1	99	98
Alpha S1	100	99
Beta S1	100	90
Gamma RBD	97	90
Delta RBD	95	98.7
Delta Spike Trimer	97	100
Epsilon RBD	100	95
Kappa RBD	99	93
D614G S1	100	99
E484K RBD	99	89
K417N RBD	96	99
N501Y RBD	99	99

* Clinical specificity was determined by testing 118 and 114 (for Delta) SARS-CoV-2–negative samples that were collected earlier than December 2019.
** Clinical sensitivity was determined by running 84 and 76 (for Delta) SARS-CoV-2–positive serum and plasma samples that were confirmed to be human IgG anti–SARS-CoV-2 positive.
n, number of samples run; RBD, receptor binding domain; S1, spike 1.

Cross-Reactivity Results

Antibody-Positive Sera	Number of Samples	Positive	Negative
HIV	1	0	1
HTLV	1	0	1
Hepatitis B	1	0	1
Hepatitis C	1	0	1
CMV	3	0	3
EBV	1	0	1
WNV	1	0	1
TOXO	1	0	1*
HSV	1	0	1
Influenza A	2	0	2
Influenza B	2	0	2
RSV	2	0	2

* Gamma RBD was slightly above the cutoff value for the TOXO sample run.
CMV, cytomegalovirus; EBV, Epstein-Barr virus; HIV, human immunodeficiency virus; HSV, herpes simplex virus; HTLV, human T-lymphotropic virus; RSV, respiratory syncytial virus; TOXO, *Toxoplasma*; WNV, West Nile virus.



Quantitative* Bio-Plex Pro SARS-CoV-2 competitive assay format and incubation times. MFI, median fluorescence intensity; δRBD, Delta receptor binding domain; αS1, Alpha spike 1; SA-PE, streptavidin-phycoerythrin.
* The Bio-Plex Pro SARS-CoV-2 Delta RBD and Delta Spike Trimer Assays are qualitative.

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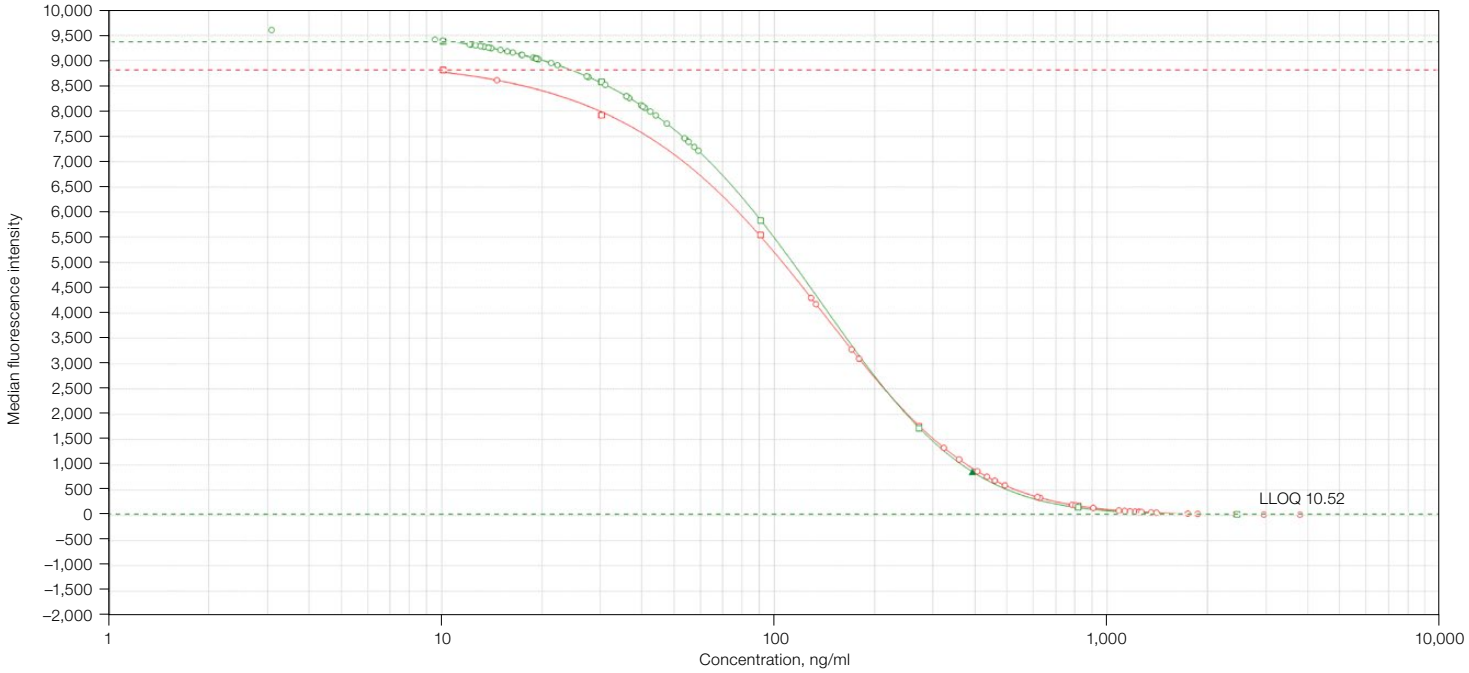
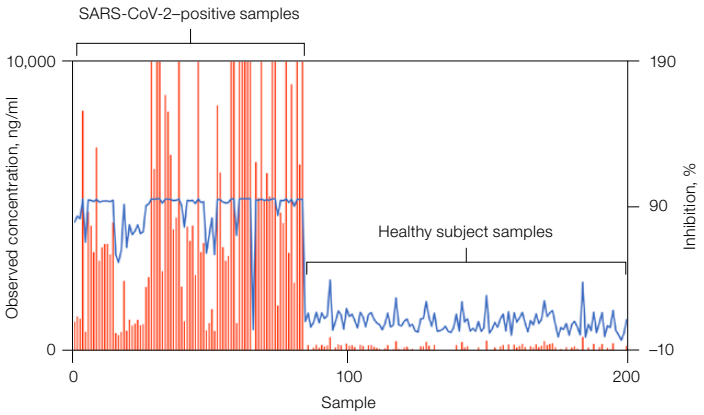
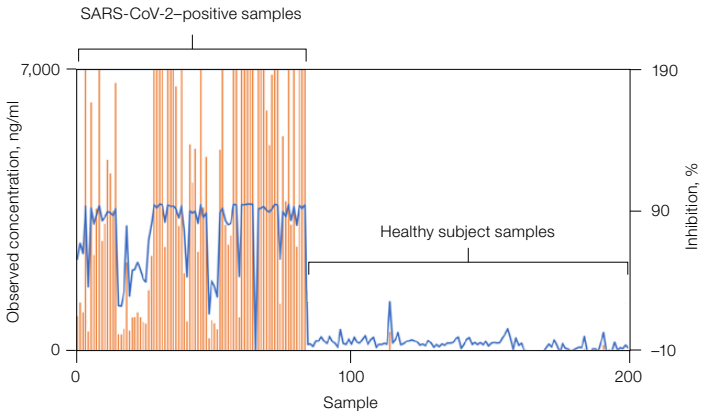
Neutralizing Antibody and Serology Assay Results



Standard curves* for SARS-CoV-2 K417N RBD with SARS-CoV-2–positive samples (n = 29) in red, healthy subject samples (n = 39) in green, and the assay positive control. The MFI signal is inversely proportional to the concentration of neutralizing antibodies in this competitive assay format. The standard calibration curve was plotted with standard points (□, □); control (▲); samples (○, ○); upper limit of quantitation (═); and lower limit of quantitation (---). * Data shown are two different experiments (SARS-CoV-2–positive samples and healthy subject samples) presented in standard curve overlay view from Bio-Plex Data Pro Software.



A plot comparing the degree of RBD antibody neutralization (percentage inhibition) and observed concentration in samples. Left, samples positive (n = 84) for SARS-CoV-2 (confirmed by PCR testing); **right**, control samples from healthy subjects (n = 118). Observed concentration (—); percentage inhibition (—).



A plot comparing the degree of S1 antibody neutralization (percentage inhibition) and observed concentration in samples. Left, samples positive (n = 84) for SARS-CoV-2 (confirmed by PCR testing); **right**, control samples from healthy subjects (n = 118). Observed concentration (—); percentage inhibition (—).

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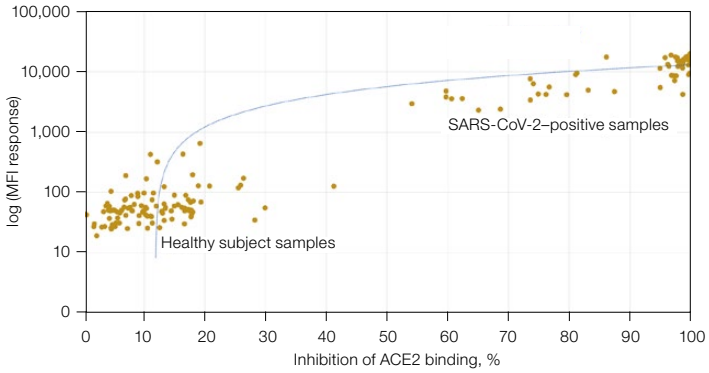

A plot that correlates the level of anti-RBD neutralizing antibody assayed using the Bio-Plex Pro Human IgG SARS-CoV-2 N/RBD/S1/S2 4-Plex Panel with the degree of neutralization (percentage inhibition) in samples. Left, control samples from healthy subjects; **right**, samples positive for SARS-CoV-2 (confirmed by PCR testing). MFI, median fluorescence intensity.

Ordering Information

Catalog #	Description
Multiplex Kits	
12016897	**Bio-Plex Pro Human SARS-CoV-2 Variant Neutralization Antibody 11-Plex Panel**, 1 x 96-well
12016848	**Bio-Plex Pro Human SARS-CoV-2 Neutralization Antibody 2-Plex Panel**, 1 x 96-well
Singleplex Variants*	
12016868	**Bio-Plex Pro SARS-CoV-2 Alpha S1 Coupled Beads**
12016849	**Bio-Plex Pro SARS-CoV-2 Beta S1 Coupled Beads**
12016898	**Bio-Plex Pro SARS-CoV-2 Gamma RBD Coupled Beads**
12017225**	**Bio-Plex Pro SARS-CoV-2 Delta RBD and Spike Trimer 2-Plex Coupled Beads**
12016875	**Bio-Plex Pro SARS-CoV-2 Epsilon RBD Coupled Beads**
12016850	**Bio-Plex Pro SARS-CoV-2 Kappa RBD Coupled Beads**
12016838	**Bio-Plex Pro SARS-CoV-2 D614G S1 Coupled Beads**
12016943	**Bio-Plex Pro SARS-CoV-2 E484K RBD Coupled Beads**
12016942	**Bio-Plex Pro SARS-CoV-2 K417N RBD Coupled Beads**
12016869	**Bio-Plex Pro SARS-CoV-2 N501Y RBD Coupled Beads**
Reagents	
12016944	**Bio-Plex Pro Biotinylated Detection ACE2 Receptor**
12016945	**Bio-Plex Pro SARS-CoV-2 Neutralization Antibody Standard**
12016837	**Bio-Plex Pro Serology Beads Storage Buffer**
Custom Assay Development Reagents	
12017037	**Bio-Plex Pro Human SARS-CoV-2 Neutralization Antibody Reagent Kit**
17007632	**Bio-Plex Pro Human SARS-CoV-2 Neutralization Antibody Custom Assay Developer Kit**

* Use any mixture of singleplex variant coupled beads in combination with the Bio-Plex Pro Human SARS-CoV-2 Neutralization Antibody 2-Plex Panel or combine the coupled beads with Bio-Plex Pro Biotinylated Detection ACE2 Receptor, SARS-CoV-2 Neutralization Antibody Standard, and Human SARS-CoV-2 Neutralization Antibody Reagent Kit.

** 12017225 comes with a vial of premixed beads with Delta RBD and Delta Spike Trimer plus a Delta positive control.

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