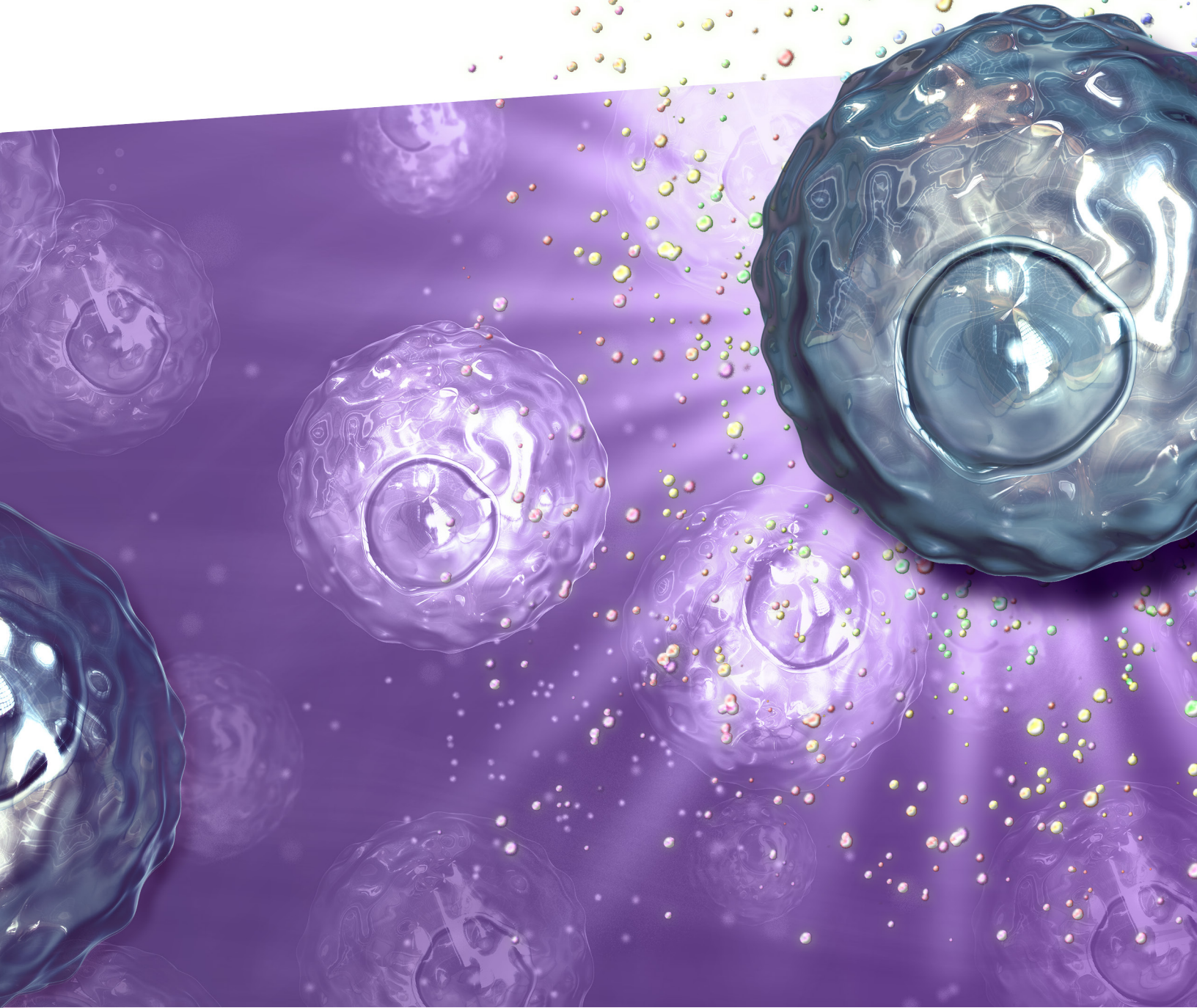


# Immunoassays

LEGENDplex™ Panels and ELISA Kits



Deciphering the immune response using cytokine detection and biomarker identification is key to advancing vaccines, infectious disease, and autoimmune disease research. Designed with the in-house expertise of our scientists, our immunoassays provide a powerful tool for the specific and sensitive quantification of these soluble proteins in biological samples. For two decades, researchers have trusted our immunoassays to help solve their most important questions. Whether your goals are specific or broad, discover how BioLegend's solutions accelerate your path to discovery.

Explore BioLegend immunoassays: [biolegend.com/en-us/bmia](https://www.biolegend.com/en-us/bmia)

## Table of Contents

LEGENDplex™ Multi-Analyte Flow Assay Kits .....	3
Workflow .....	3
Features and Specifications .....	4
Products .....	4
Featured Kits.....	5
Validation Testing and Quality Control .....	5
Additional Reagents .....	6
ELISA Kits and Sets .....	7
Types of ELISAs .....	7
ELISA Kits .....	8
Validation Testing and Quality Control .....	9
Featured Products: Novel Kits to Dismantle SARS-CoV-2.....	10
Additional ELISA Reagents .....	11
Mini-ELISA Plate Reader™ .....	11

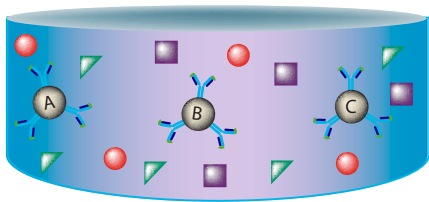
# LEGENDplex™ Multi-Analyte Flow Assay Kits

LEGENDplex™ bead-based immunoassays work on the same principle as a sandwich ELISA, with the advantage that they can simultaneously quantify up to 14 targets in a single sample using only a flow cytometer—no other instruments are needed. Data can be analyzed with our free LEGENDplex™ Data Analysis Software Suite. Choose from dozens of existing panels or customize your own.

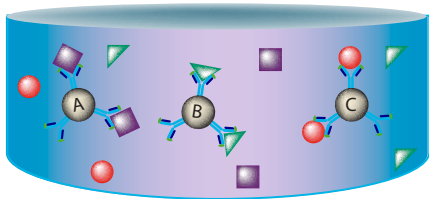
View our full line of LEGENDplex™ panels at: [biolegend.com/en-us/legendplex](http://biolegend.com/en-us/legendplex)

## Workflow

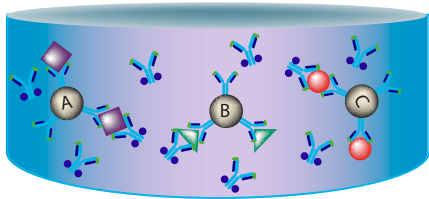
See kit manual for details specific to filter or V-bottom plates.



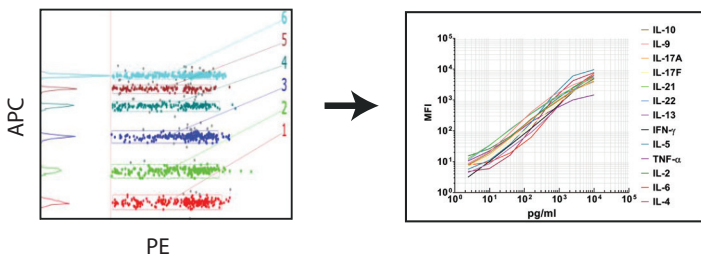
Incubate capture beads with a biological sample, such as serum, plasma, or cell culture supernatant. Capture beads are conjugated to analyte-specific antibodies and can be differentiated by size and internal APC fluorescence intensity.



Add the biotinylated detection antibody cocktail to form bead-analyte-detection antibody complex.



Add the streptavidin-PE conjugate to bind to the biotinylated detection antibodies.



Collect data using a flow cytometer. The streptavidin-PE conjugate will provide a fluorescent signal intensity proportional to the amount of bound analyte.

Quantify the concentrations of analyte targets using LEGENDplex™ data analysis software.

## Features and Specifications

LEGENDplex™ assays work with any flow cytometer capable of detecting PE and APC fluorescence, or PE and PerCP in the case of instruments without a red laser. No other instruments are needed.

In addition, we offer FREE data analysis software. The data analysis software suite is designed to analyze LEGENDplex™ flow cytometry data files. It consists of both an online cloud-based program and a downloadable desktop application for both PC and Mac.

### LEGENDplex™ software features include:

- Simple, intuitive user interface and workflow
- Pre-loaded LEGENDplex™ assay bead IDs
- A sophisticated auto-gating algorithm
- Simple import of experiment workspaces between cloud-based program and desktop applications

Access LEGENDplex™ software: [biolegend.com/en-us/legendplex#software](http://biolegend.com/en-us/legendplex#software)

## Products



LEGENDplex™ assays are available for human, non-human primate (NHP), mouse, and rat specificities. Assays can be ordered as Pre-defined panels, Mix and Match panels, or customized panels.

**Pre-defined Panels:** The panels provide all of the reagents needed to run the assay, including setup beads, pre-defined capture beads, matrix, detection antibodies, standard cocktail, buffers, assay plate, and plate sealers.

**Mix and Match Assays:** Ideal for users that only need to assess a subset of analytes within a pre-defined panel. With the Mix and Match option, you will need to order your desired capture beads, the appropriate buffer set, standard cocktail, detection antibody mix, and assay plate format (filter or V-bottom). See Table 1 that illustrates Pre-defined and Mix and Match panel features.

**Custom Panels:** Want a LEGENDplex™ target that you don't see in our panel listings? Or, perhaps you want to combine cytokines and chemokines across panels? Just choose your desired combination of analytes and our scientists will contact you and help design a Custom LEGENDplex™ panel to suit your research needs.

Get started at [biolegend.com/legendplex#custompanels](http://biolegend.com/legendplex#custompanels)

Table 1: Example of Pre-defined and Mix and Match Panels

Pre-defined Panels include ALL required components and ALL check-marked targets				Mix and Match Capture Beads Cat. No.
Filter Plate		Cat. No. 740349	Cat. No. 740350	
V-bottom Plate		Cat. No. 740390	Cat. No. 740396	
Target	Bead ID	Anti-Virus Response Panel	Type 1/2/3 Interferon Panel	
CXCL10 (IP-10)	A7	✓		740363
GM-CSF	B5	✓		740362
IFN-α2	B3	✓	✓	740351
IFN-β	B6	✓	✓	740353
IFN-γ	B9	✓	✓	740352
IFN-λ1	A8	✓	✓	740354
IFN-λ2/3	B4	✓	✓	740355
IL-1β	A4	✓		740119
IL-6	A5	✓		740356
IL-8	A10	✓		740357
IL-10	B7	✓		740358
IL-12 (p70)	B2	✓		740361
TNF-α	A6	✓		740359
Required Mix and Match Components (already included in Pre-defined Panel)				
Standard			Cat. No. 740365	
Detection Antibodies			Cat. No. 740364	
Buffer Set A			Cat. No. 740368	
Plate Options			Filter Plate: Cat. No. 740377	
			V-bottom Plate: Cat. No. 740379	

## Features

No. analytes/assay	1 to 14
Min. volume	12.5 µL serum/plasma; 25 µL tissue culture supernatant
Cytometer requirements	PE & APC detection (PerCP/FL3 without red laser)
Analysis software	Free downloadable and cloud-based versions for both Mac and PCs
Tests/assay	100 tests

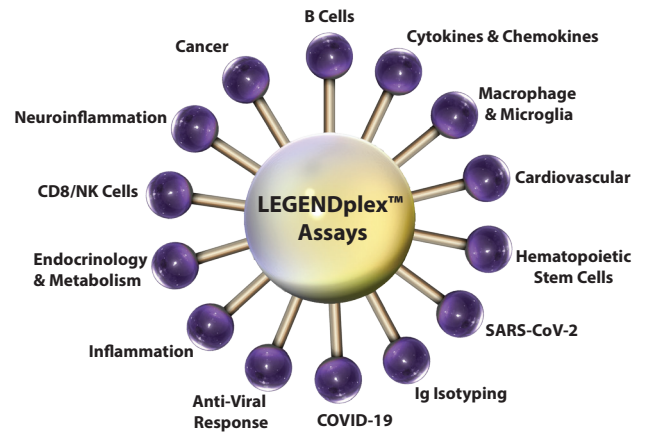
### Cloud-specific features:

- Access from any web-connected computer
- 2 GB of storage space per user account
- One-click access to previously uploaded data
- Easily share results with other users

## Featured Kits

We have developed LEGENDplex™ panels for use in a wide variety of research fields in the scientific community. Each LEGENDplex™ panel is specifically designed to quantitate analytes that are central to a given cell type, biological process, or disease state. We are constantly expanding our catalog with new assay content.

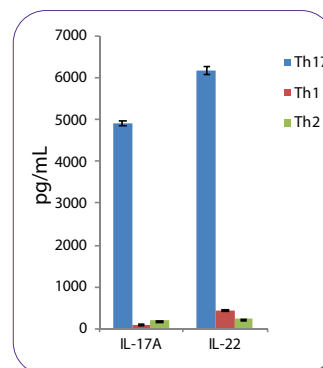
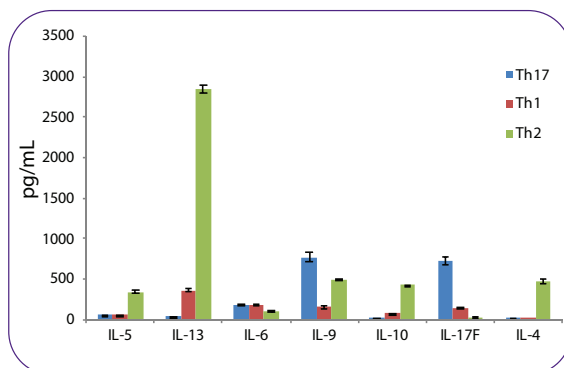
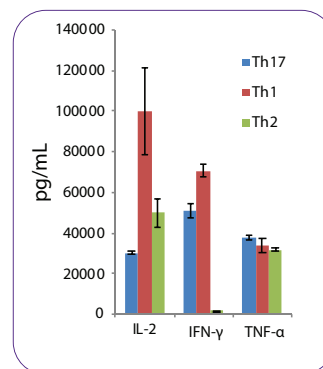
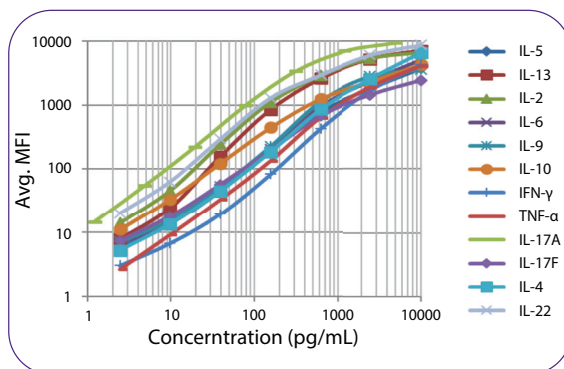
- Our COVID-19 Cytokine Storm panels can help examine cytokine dysregulation associated with the clinical progression of COVID-19 disease.
- We offer an array of panels to profile immunological responses of B cell and T helper cell functions, CD8/Natural Killer cell activities, and additional cell types.
- We also offer panels to examine factors involved in cardiovascular health and metabolic disorders.



## Validation Testing and Quality Control

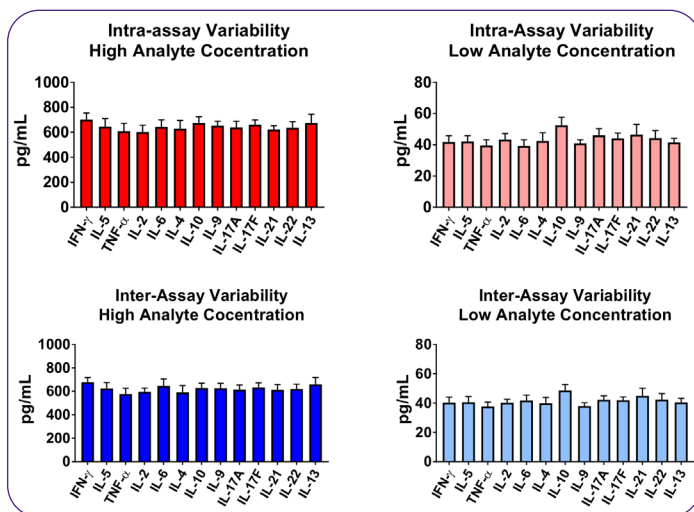
### Dynamic Range and Sensitivity

In this representative study, data generated from the LEGENDplex™ Human T Helper Cytokine Panel were analyzed using our free LEGENDplex™ Data Analysis Software Suite and used to construct standard curves for all analytes in the multiplexed panel, showing both the dynamic range and sensitivity of the assay.



PBMC derived naive CD4<sup>+</sup> T cells were differentiated under Th1, Th2 or Th17 conditions for 7 days. The cells were then restimulated with Cell Activation Cocktail (without Brefeldin A) for 16 hours. Cell culture supernatant was collected and measured with LEGENDplex™ Human T Helper Cytokine Panel.

## Precision and Reproducibility



LEGENDplex™ assays yield precise and highly reproducible data. The top panel (red bars) exhibits intra-assay precision for two samples with different concentrations of target proteins analyzed in a single assay with 16 replicates for each sample. The bottom panel (blue bars) exhibits inter-assay precision and reproducibility for the same two samples with different concentrations of target proteins analyzed in three independent assays (3 replicates/sample).

## Additional Reagents

### Carboxyl Beads (Unconjugated)

Can't find a LEGENDplex™ bead for the target you want? Now you can conjugate your antibody of interest to our unconjugated carboxyl beads. Bead IDs are provided so you can fit them appropriately into your LEGENDplex™ panel. If you would like advice regarding the addition of these beads to your panel, talk with us at: [cst@biolegend.com](mailto:cst@biolegend.com).

Beads	Cat. No.
A4	740167
A5	740168
A6	740169
A7	740170
A8	740171
A10	740172
B2	740173
B3	740174
B4	740175
B5	740176
B6	740177
B7	740178
B9	740179

### Streptavidin-PE Reporter Reagent

Streptavidin binds to biotin with high affinity. Streptavidin-PE (SA-PE) is useful for detecting biotinylated antibodies. It is used in LEGENDplex™ assays as a reporter reagent. PE provides a strong signal that can be detected on most common flow cytometers.

Description	Cat. No.
SA-PE	740452

# ELISA Kits and Sets

Focus on a single target with our ELISA kits and sets. We offer four types of ELISA kits, including RAPID MAX™ kits that cut your assay time down to less than 90 minutes, easy-to-use LEGEND MAX™ kits containing pre-coated plates, and economical sets with the required components to develop your own ELISA.

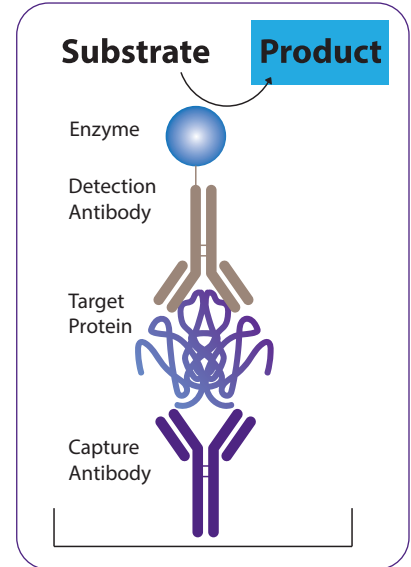
Find your ELISA kits, sets, and reagents at: [biolegend.com/en-us/elisa](https://www.biolegend.com/en-us/elisa)

## Types of ELISAs

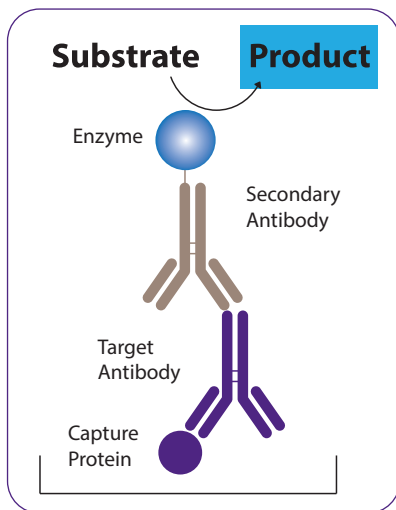
### Sandwich ELISA

A sandwich ELISA is ideal for analyzing soluble proteins like cytokines and growth factors in complex samples. Sandwich ELISAs typically utilize plates, coated with capture antibodies, to immobilize soluble proteins. The bound proteins are then recognized by a second antibody, known as the detection antibody, which is often labeled with an enzyme. Finally, a colorimetric substrate is added, which results in a color change that is proportional to the amount of antigen captured. By using a plate reader to measure this signal and plotting the resulting values against a standard curve with known quantities of analyte, you can obtain precise, quantitative measurements.

A typical sandwich ELISA can take at least 22 hours as it includes coating the plate with antibody overnight and numerous wash steps. We offer different types of ELISA kits to streamline this workflow, thereby reducing the number of protocol steps and saving you time.



### Indirect ELISA



Indirect ELISA-based methods are useful when you want to measure antibody concentration in a biological sample. Indirect ELISAs typically utilize a plate coated with the antigen (or capture protein) to immobilize the antibody in question. Next, a secondary enzyme-conjugated antibody is applied. Finally, a colorimetric substrate is added. Like a sandwich ELISA, you can use a plate reader to detect the signal and analyze by plotting these values against a standard curve with known quantities of analyte.

Some of our ELISA kits and sets, including those designed to detect anti-SARS-CoV-2 antibodies, utilize an indirect ELISA-based workflow.

## ELISA Kits

For increased flexibility and convenience, we provide four types of ELISA kits suitable for both experts and beginners. Each of our ELISA kits is designed for the quantification of proteins or antibodies in biological samples including cell culture supernatant, serum, and plasma. They offer high accuracy, sample linearity, and testing on biologically relevant samples.



### RAPID MAX™ ELISA Kits

Our RAPID MAX™ kits cut assay time down to less than 90 minutes. Each kit is fully validated and contains a 96-well strip plate that has been pre-coated to immobilize the biotinylated capture antibody or protein. Using a conjugated detection antibody and fewer wash steps, this ELISA is designed to give accurate results in less time.



### LEGEND MAX™ ELISA Kits

These ready-to-use kits contain all of the required reagents, including a 96-well strip plate pre-coated with capture antibody. The kits are validated in both normal serum and plasma and biologically relevant samples, and the manuals provide additional information regarding recovery and linearity.



### ELISA MAX™ Deluxe Sets

ELISA MAX™ Deluxe sets are a cost-effective alternative to our other kit formats. These contain pre-optimized reagents and some additional buffers and solutions. Plates are sold separately and will need to be coated with capture antibody by the end-user.



### ELISA MAX™ Standard Sets

ELISA MAX™ Standard sets provide only the basic components to complete an ELISA, including a recombinant protein standard, Avidin-HRP, and capture and detection antibodies. This set is perfect for those who want to stretch their budget by supplying their own buffers and coating their own plates.

Components	RAPID MAX™ Kit	LEGEND MAX™ Kit	ELISA MAX™ Deluxe Set	ELISA MAX™ Standard Set
Assay Time	90 mins	4 hours	Overnight + 6 hours	Overnight + 6 hours
Experience Level	Beginner-Advanced	Beginner-Advanced	Intermediate-Advanced	Intermediate-Advanced
Strip-Well Plate	✓	✓		
Capture Antibody or Protein	✓	Pre-coated on plate	✓	✓
Detection Antibody	✓	✓	✓	✓
Protein or Antibody Standard	✓	✓	✓	✓
Avidin-HRP	Not required	✓	✓	✓
Coating Buffer	Not required	Not required	✓	
Wash Buffer	✓	✓		
Assay Buffer/Assay Diluent/Matrix	✓	✓	✓	
Substrate/TMB Solution	✓	✓	✓	
Stop Solution	✓	✓		
Plate Sealers	✓	✓		



## Validation Testing and Quality Control

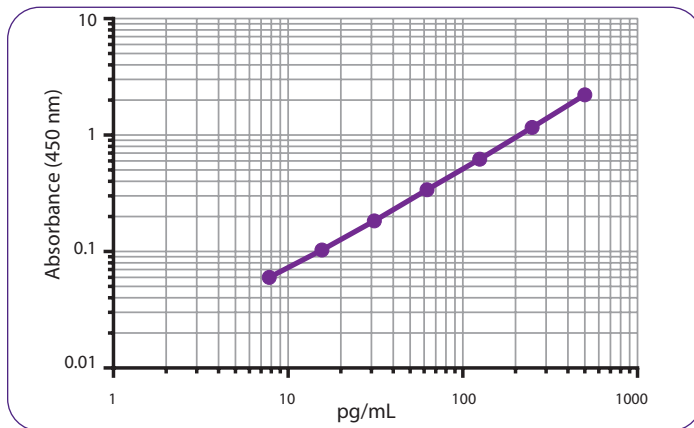
Our ELISA kits undergo validation testing and quality control experiments to ensure they provide specificity, sensitivity and excellent dynamic range. We provide validation data, including the minimal detectable concentration, for our LEGEND MAX™ and RAPID MAX™ kits.

To ensure that the ELISA detects only the protein of interest, antibody reagents are tested for cross-reactivity against other proteins, isoforms, and multimers (if applicable). Antibody reactivity to orthologous proteins in other species is also assessed.

### Precision and Reproducibility

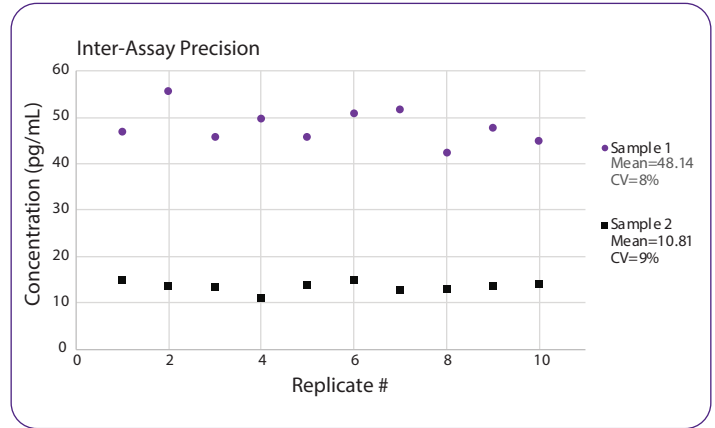
For our LEGEND MAX™ and RAPID MAX™ kits, we provide intra-assay and inter-assay precision statistics from replicates within one plate and from plate-to-plate to ensure reproducibility.

#### LEGEND MAX™ Mouse IFN- $\beta$ ELISA Kit



Representative data for a standard curve using the LEGEND MAX™ Mouse IFN- $\beta$  ELISA Kit (Cat. No. 439407).

#### LEGEND MAX™ Human PD-L2 ELISA Kit

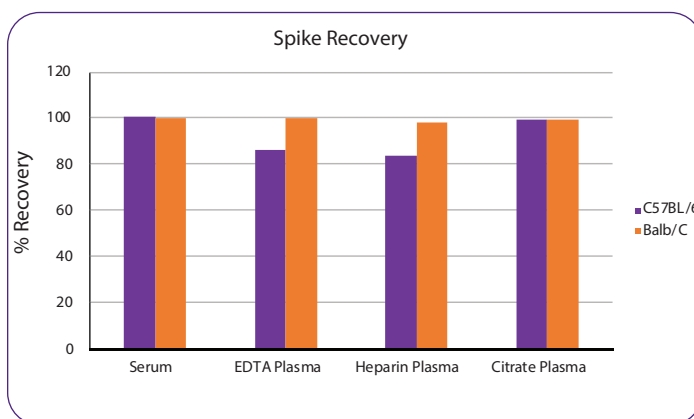


Two samples with different PD-L2 concentrations were tested for intra- and inter-assay precision using the LEGEND MAX™ Human PD-L2 ELISA Kit.

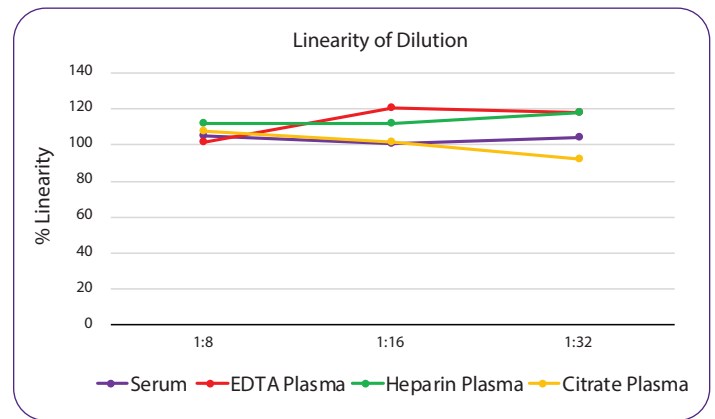
### Spike Recovery and Linearity of Dilution

The recovery characteristics of an ELISA can be described by the ability to accurately measure different quantities of a standard spiked into sample matrices. We collect recovery data for all LEGEND MAX™ and RAPID MAX™ kits, typically tested in serum, plasma, and cell culture supernatant. These kit formats also provide results regarding linearity of dilution, which indicate that the dose-response to an analyte's concentration is linear and within the range of the assay standard curve.

#### LEGEND MAX™ Mouse CXCL1 ELISA Kit



Serum and plasma from C57BL/6 and Balb/C mice were diluted 1:4 and recombinant mouse CXCL1 was added to the samples. CXCL1 protein was quantified using the LEGEND MAX™ Mouse CXCL1 ELISA Kit.

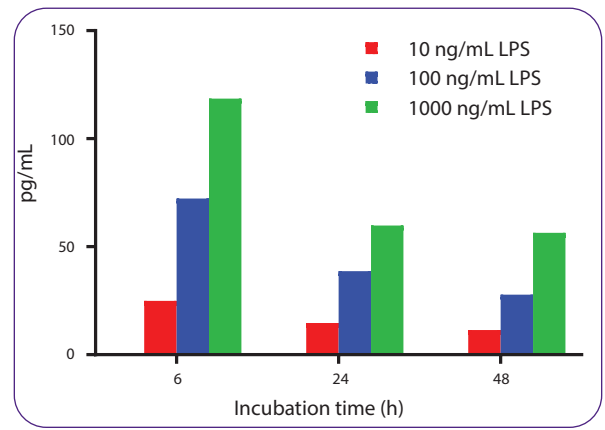


Samples were diluted to the recommended dilution factor (1:4), spiked with recombinant CXCL1, and further diluted in a series of 2-fold dilutions. The percent linearity was averaged for each sample type.

## Biological Sample Detectability

All of our ELISA kits and sets are designed for quantification of proteins in cell culture supernatant, serum, plasma, and other biological samples. LEGEND MAX™ and RAPID MAX™ kit manuals contain results from testing of conditional media, using biologically relevant samples and conditions in which the expected cytokine or chemokine is produced. Normal serum and plasma values are also provided.

## IFN-β Production by RAW 264.7 Cells



RAW 264.7 cells were plated in a 12-well plate at  $3 \times 10^6$  cells/well until 80% confluent, then stimulated with various concentrations of LPS. Supernatants were removed at various time points and assayed using the LEGEND MAX™ Mouse IFN-β ELISA Kit (Cat. No. 439407).

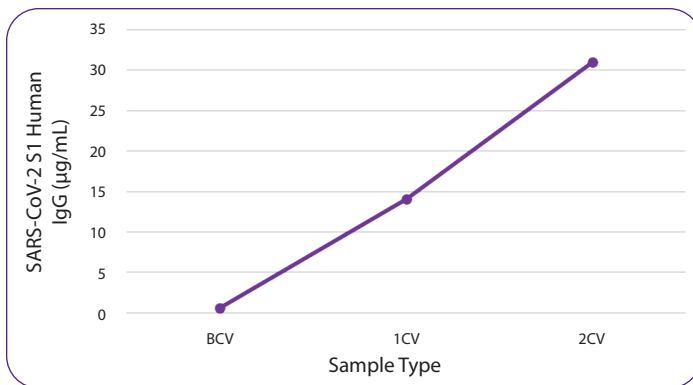
## Featured Products: Novel Kits to Dismantle SARS-CoV-2

We offer ELISA kits to detect targets in human, mouse, and rat samples across a variety of research areas including immunology, oncology, stem cell biology, and neuroscience. They can be used to assess the immune environment and its inflammatory state for vaccine, infectious disease, and autoimmune disease research.

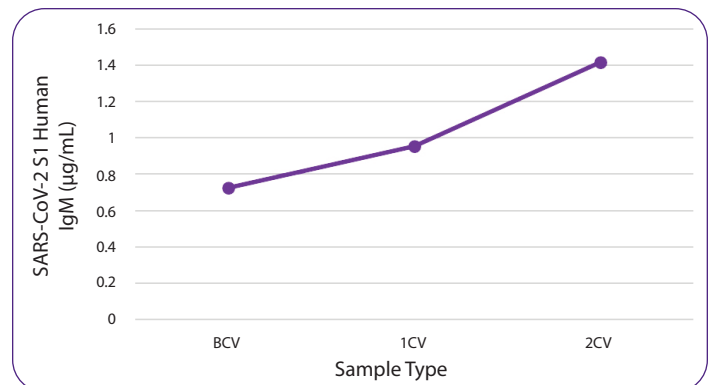
Accelerate COVID-19 research by dismantling the virus' key components with our reagents. We provide ELISA kits to track cytokine response and to quantify relevant SARS-CoV-2 antibodies and proteins, including kits to detect anti-spike protein antibodies and nucleocapsid protein.

To validate the performance of our COVID-19 ELISA kits, we measured anti-SARS CoV-2 antibodies in serum (n=26) collected prior to vaccination (BCV), two weeks following the first dose (1CV), and two weeks following the second dose (2CV) of either the Moderna or Pfizer SARS-CoV-2 vaccines.

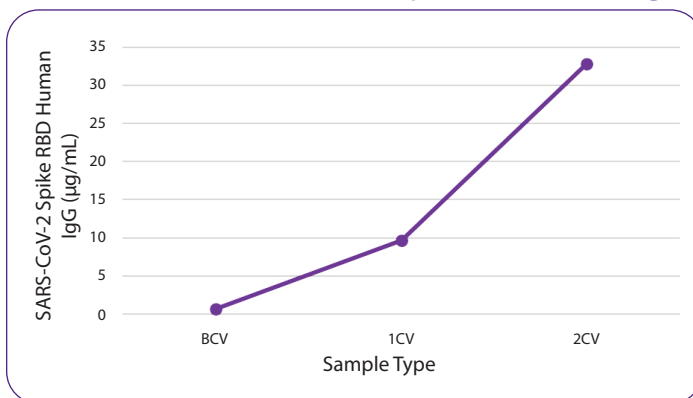
### LEGEND MAX™ SARS-CoV-2 Spike Protein S1 IgG



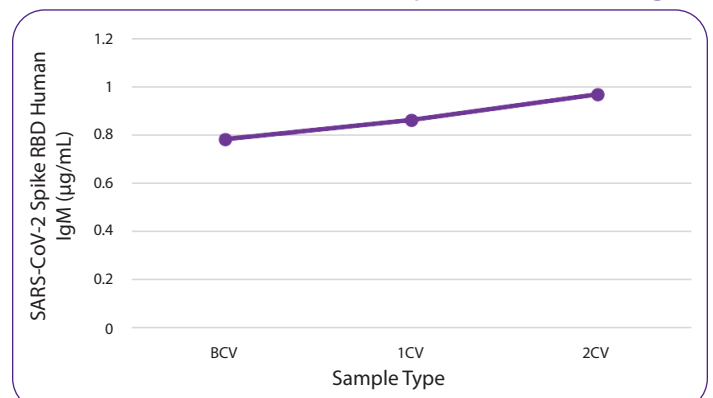
### LEGEND MAX™ SARS-CoV-2 Spike Protein S1 IgM



### LEGEND MAX™ SARS-CoV-2 Spike Protein RBD IgG



### LEGEND MAX™ SARS-CoV-2 Spike Protein RBD IgM



## Additional ELISA Reagents

For increased flexibility, we provide separate antibody pairs and recombinant protein standards as single reagents that are validated for sandwich ELISA assays. Each antibody pair consists of a purified capture antibody and biotinylated detection antibody.

Explore ELISA antibody pairs: [biolegend.com/en-us/elisa-antibody-pairs](https://www.biolegend.com/en-us/elisa-antibody-pairs)

### Buffers and Plates

Assay Buffers	Cat. No.
ELISA Coating Buffer (5X)	421701
ELISA Wash Buffer (20X)	421601
Assay Diluents	
ELISA Assay Diluent (5X)*	421203
ELISA Assay Diluent B (5X)*	421205
Enzyme Conjugates	
HRP Avidin	405103
HRP Streptavidin	405210

\*ELISA Assay Diluent (5X) contains bovine calf serum. ELISA Assay Diluent B (5X) contains bovine serum albumin.

\*\*TMB High Sensitivity Substrate Solution is a one-step working solution for HRP-labeled conjugates that provides high signal and high sensitivity. The TMB Substrate Set contains substrate A and substrate B solutions that must be mixed before use.

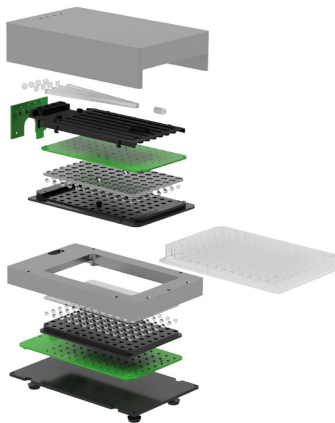
Substrate Solutions	
TMB High Sensitivity Substrate Solution**	421501
TMB Substrate Set**	421101
Stop Solution for TMB Substrate	423001
Assay Plates	
Nunc™ MaxiSorp™ ELISA Plates, Uncoated	423501
Plate Sealers	423601

## Mini-ELISA Plate Reader™

Reading your ELISA should be as streamlined as the rest of your ELISA workflow. Achieve end-to-end efficiency with BioLegend's Mini ELISA Plate Reader™ – a fast, compact instrument that saves you time and space in the lab. A single USB cord enables plug-and-play functionality, and 96 detection units scan all 96 wells simultaneously. Intuitive operation makes it easy to read your plate and obtain data at a rapid pace.



Learn more and watch a tutorial video: [biolegend.com/en-us/elisa-plate-reader](https://www.biolegend.com/en-us/elisa-plate-reader)



### Features:

- Read a plate in under 5 seconds
- Compact design for better handling and portability.
- No moving parts make this instrument maintenance-free.
- Four standard filters – 405, 450, 492, and 620 nm – allow for the measurement of a variety of substrates and endpoint and kinetic assays.

### Save Space Without Sacrificing Performance

Human serum, plasma, and cell culture supernatant samples (n = 32) were assayed using LEGEND MAX™ Human PTX3 ELISA Kit (Cat. No. 446807). Optical density (O.D.) was read using a standard plate reader at 450 nm with wavelength correction set to 570 nm and the BioLegend Mini ELISA Plate Reader™ at 450 nm with wavelength correction set to 620 nm. Sample concentrations (pg/mL) were calculated and plotted for sample value correlation.

### BioLegend Mini ELISA Plate Reader™ vs. Regular Plate Reader

