Time-saving immunoassay tools

Instant ELISA kits, ProQuantum high-sensitivity immunoassays, and ProcartaPlex multiplex panels.

For over 40 years, the well-established enzyme-linked immunosorbent assay (ELISA) has been considered the gold standard for target-specific protein measurement in classic immunology studies. With reagents conveniently packaged in ready-to-use or coat-it-yourself kits, microplate-based ELISAs offer a larger selection of targets than any other immunoassay platform available, as well as a long list of published references that validate their use. Despite the useful kit formats, however, it can still take weeks or months to optimize a working protocol and complete the experimental assay runs needed for a research study. With an increased urgency to understand and develop treatments for a multitude of devastating human diseases, today's life science researcher needs assay platforms that can quantify proteins with the same sensitivity and specificity as conventional ELISAs but in a faster and easier way.

The Invitrogen™ family of immunoassays offers a number of tools with which to explore all facets of the immune system and to monitor changes in immune responses for pathophysiological conditions. In addition to conventional ELISAs, we have developed Instant ELISA™ kits, ProQuantum™ high-sensitivity immunoassays, and ProcartaPlex™ multiplex panels, which provide advanced features to support time savings and increased productivity in the lab (Figure 1, Table 1).

Instant ELISA kits provide the same specificity and sensitivity as ELISAs but with less hands-on time

In a typical sandwich ELISA, the antigen of interest is immobilized on a solid surface via capture by an antigen-specific antibody that has been precoated on the wells of a microplate. The bound antigen is then quantified after binding with a second antigen-specific antibody that is linked to an enzyme (direct ELISA) or that is detected with either an enzyme-linked secondary antibody or a biotinylated secondary antibody in conjunction with enzyme-linked streptavidin (indirect ELISA). The amount of antigen is reported by determining the conjugated enzyme's activity after incubation with a substrate that produces a measurable product.

In contrast to conventional ELISA kits, Instant ELISA kits come with plates that contain more than just the precoated capture antibody. The 96-well plates in the Instant ELISA kits contain lyophilized detection antibody, streptavidin-HRP, and sample diluent, greatly reducing pipetting time during the assay. These kits also provide four additional

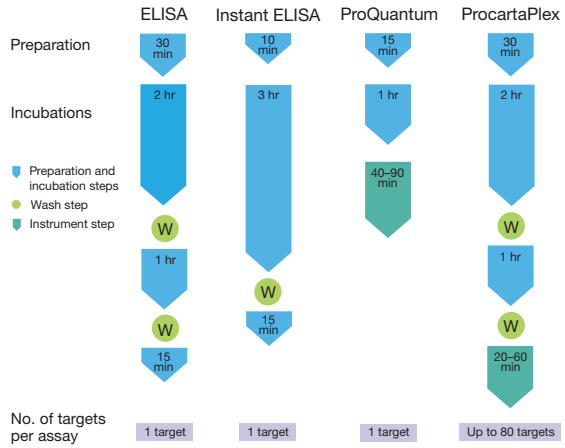

Figure 1. Comparison of the workflows for the Instant ELISA kit, ProQuantum immunoassay, and ProcartaPlex multiplex panel with that of a conventional ELISA. The Invitrogen™ Instant ELISA™ protocol saves hands-on time with simplified preparation steps and a single wash step, compared with that of a conventional ELISA. The Invitrogen™ ProQuantum™ immunoassays feature a streamlined workflow and consume as little as 2 µL sample per result. The Invitrogen™ ProcartaPlex™ multiplex panels can provide the same information as up to 80 individual ELISAs.

Table 1. Comparison of the protocol and assay requirements of a conventional ELISA with those of three alternative ELISAs.

	ELISA	Instant ELISA	ProQuantum	ProcartaPlex
Time-to-result	4 hr	4 hr	2 hr	4.5 hr
Hands-on time	80 min	40 min	40 min	80 min
Wash steps	Yes, multiple	One wash	No wash	Yes, multiple
Multiplexing	No	No	No	Yes, up to 80 targets
Sample volume	50–100 µL	50–100 µL	2–5 µL	25–50 µL
Readout	HRP-TMB	HRP-TMB	FAM (fluorescence)	RPE (fluorescence)
Instrument	Microplate reader	Microplate reader	qPCR instrument	Luminex® instrument
Instrument read time	2 min	2 min	40–90 min	20–60 min

8-well strips of lyophilized, serially diluted protein standards that are ready to use. Therefore, each Instant ELISA kit can run more samples (96 tests, plus 4 strips or 32 wells of standards) than a conventional ELISA kit, and no plate setup or serial dilutions are required. Simply reconstitute the reagents in the wells with water, and add your unknown samples. After a 3-hour incubation and one wash step, add the enzyme substrate and then end the reaction with stop solution. With the Instant

ELISA workflow, both preparation time and hands-on time are much shorter than with a conventional ELISA. Moreover, instead of attending to your plate every hour to do washes, you have a 3-hour window to focus on something else before wrapping up the assay run. Learn more about our ELISA kit formats, including the Instant ELISA kit, at thermofisher.com/instantelisa.

ProQuantum immunoassays use qPCR to provide a next-generation alternative

Our newest immunoassay platform innovation—the ProQuantum high-sensitivity immunoassay—features a streamlined workflow with no wash steps and consumes as little as 2 µL of sample per result. The small sample-volume requirement means less work, not just during the assay run itself but also during sample collection procedures. In addition, the readout of the ProQuantum immunoassay is on a qPCR instrument, leveraging the high sensitivity and large dynamic range of qPCR technology. The ProQuantum immunoassays can quantify analytes over a concentration range of up to 5 orders of magnitude or more, minimizing the need for sample dilutions.

ProQuantum immunoassays are similar to ELISAs in that they both use matched antibody pairs to detect the target analyte (Figure 2). The assay is based on an optimized pair of antibodies that bind to specific epitopes in close proximity on the analyte. These antibodies are pre-conjugated at the 3´ end of a 60-base oligonucleotide or the 5´ end of a 40-base oligonucleotide (Figure 2A). When added to a sample suspension containing the specific analyte to be quantified, the two antibodies bind to their respective epitopes, which results in the two oligonucleotide strands being brought into proximity of one another (Figure 2A). This antibody binding provides structural stability such that in the presence of DNA ligase and a third splint oligonucleotide (complementary to the ends of the other two DNA oligonucleotides), the two antibody-conjugated oligonucleotides are ligated together to create a 100-base strand that can serve as a DNA amplification template (Figure 2B). Following a temperature increase to 95°C to inactivate the ligase and denature the analyte proteins and antibodies, the template is amplified through 40 cycles of Applied Biosystems™ TaqMan® fluorescence-based qPCR. The amount of amplification after each cycle (fluorescence increase, see Figure 2C) is directly proportional to the number of ligated templates created by the antibody–analyte binding (Figure 2D).

Each ProQuantum immunoassay kit provides enough reagents to scale an assay up or down depending on the user's comfort level with

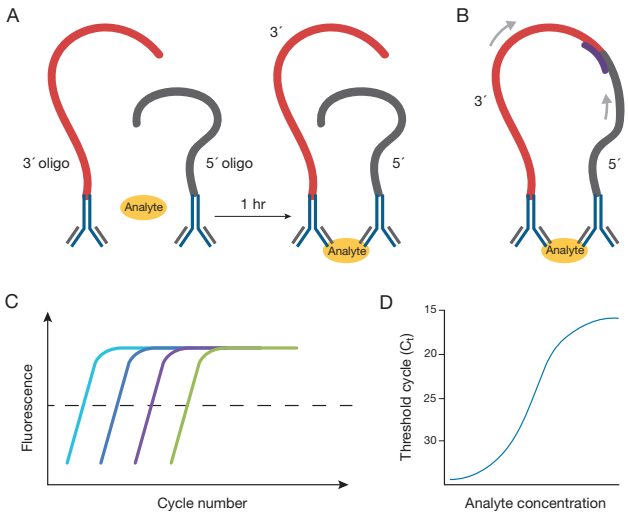

Figure 2. How ProQuantum immunoassays work. ProQuantum immunoassays utilize a matched pair of analyte-specific antibodies, each conjugated to a DNA oligonucleotide. During antibody–analyte binding, the two DNA oligonucleotides are brought into close proximity **(A)**, which then allows for ligation of the two strands by DNA ligase in the presence of a third splint oligonucleotide to create a 100-base template for amplification **(B)**. **(C)** Once the ligase is inactivated, the sample is amplified through 40 qPCR cycles, and the amount of DNA produced, measured via fluorescence, is directly proportional to the number of amplicons generated. This graph shows the fluorescence vs. cycle number curves for four different starting analyte concentrations; the dashed line represents the fluorescence threshold. **(D)** The cycle number required to reach the fluorescence threshold (threshold cycle, or C_t) is plotted vs. analyte concentration to create a standard curve.

small-volume pipetting. Run one 96-well plate if pipetting 5 µL/well, two 96-well plates if pipetting 2 µL/well, or even one 384-well plate for liquid automation handling of 1 µL/well. PCR plates are not included because they should be compatible with the qPCR instrument that will be used. In the ProQuantum protocol, we recommend using a "setup plate" to prepare and organize solutions, samples, and standards. Once the solutions and samples are prepared, use a multichannel pipette to transfer the prepared solutions from the setup plate to the actual PCR plate; this step helps to avoid evaporation when working with small volumes. Incubate the PCR plate at room temperature for 1 hour to allow for antibody–analyte binding, then add the ProQuantum master mix and ligase solutions directly to each well and place the plate into the qPCR instrument for the ligation and qPCR amplification steps. The new free ProQuantum companion software enables easy standard curve generation and data analysis and is accessible from Thermo Fisher™ Cloud. To learn more about ProQuantum immunoassays and software, visit thermofisher.com/proquantum. →

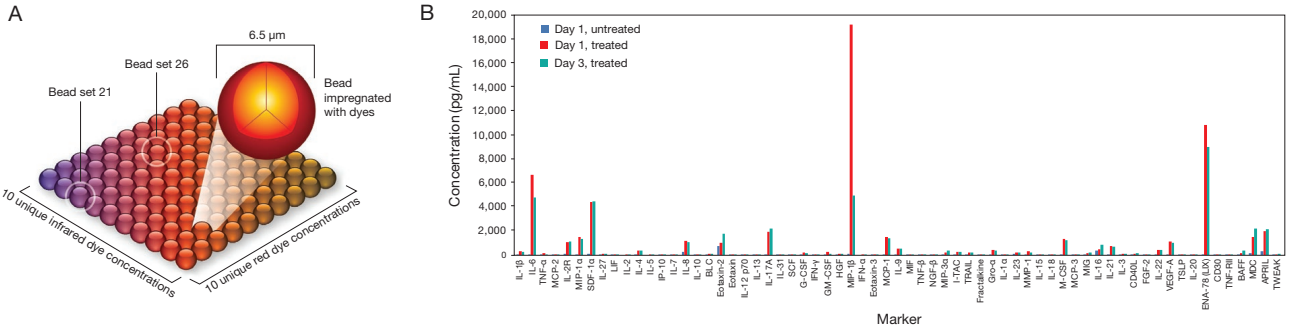

Figure 3. ProcartaPlex multiplex immunoassay system. (A) xMAP magnetic beads are color-coded to enable differentiation of proteins in a single well. **(B)** Using the Invitrogen™ Immune Monitoring 65-Plex Human ProcartaPlex™ Panel (Cat. No. EPX650-10065-901), we quantitatively analyzed 65 soluble protein biomarkers in human peripheral blood mononuclear cells (hPBMCs, collected from a single individual) before treatment, and 1 day and 3 days after treatment with lipopolysaccharide (LPS).

ProcartaPlex panels enable high-level multiplexing to screen proteins

ProcartaPlex multiplex immunoassays are antibody-based magnetic-bead reagent kits and panels for multiplex protein quantitation using the Luminex® instrument platform and associated xMAP® magnetic bead technology. The Luminex® MagPlex® superparamagnetic microsphere beads in the ProcartaPlex assays are internally dyed with precise proportions of red and infrared fluorophores to create 100 spectrally unique signatures (Figure 3A), which can be identified by the Luminex xMAP detection systems, including the Luminex® 200™, FLEXMAP 3D®, and MAGPIX® systems. Similar to a sandwich ELISA, the ProcartaPlex assay uses matched antibody pairs to identify the protein of interest. In a ProcartaPlex multiplex assay, each spectrally unique bead is labeled with antibodies specific for a single target protein, and bound proteins are identified with biotinylated antibodies and streptavidin–R-phycoerythrin (RPE). The conjugation of protein-specific antibodies to a distinct bead allows for analysis of multiple analytes in a single well (Figure 3B). The biggest difference between the two types of immunoassays is that the capture antibody in the ProcartaPlex assay is conjugated to a magnetic bead and not adsorbed to the microplate well as in a conventional ELISA, and therefore the ProcartaPlex assay reagents are free-floating in the solution. For detection, the Luminex 200 instrument, for example, contains two lasers, one to distinguish the spectral signature of each bead and the second to quantify the amount of RPE fluorescence, which is proportional to the amount of protein present in the sample.

ProcartaPlex assays are designed for quantitation of more than 200 cytokine, chemokine, growth factor, and other protein targets from a variety of species (human, mouse, rat, nonhuman primate, canine, and porcine). ProcartaPlex assays allow simultaneous measurement of up to 80 proteins in a single well, which would be equivalent to running 80 individual ELISAs, and a ProcartaPlex multiplex panel can be run in the same amount of time as a single ELISA, with a very similar workflow. In addition, with a ProcartaPlex panel, only 25 µL of sample is required to screen for up to 80 proteins, providing significant sample volume savings. This approach is amenable to high-throughput screening and ideal for looking at many proteins before narrowing down to a handful of specific proteins to interrogate. Select from over 60 preconfigured multiplex panels (2- to 65-plex) or blend singleplex kits (90% are combinable) to create custom multiplex assays that can screen for up to 80 different target proteins. Learn more at **thermofisher.com/procartaplex**.

Learn more

To find out more about the Instant ELISA kits, ProQuantum high-sensitivity immunoassays, and ProcartaPlex multiplex panels and the protein targets currently available for each assay, visit **thermofisher.com/immunoassays** (where you can also download a free biomarker quantitation assay guide). ■

Selected products	Quantity	Cat. No.
Instant ELISA kits		
TNF alpha Human Instant ELISA™ Kit	128 tests	BMS223INST
IL-6 Human Instant ELISA™ Kit	128 tests	BMS213INST
ProQuantum high-sensitivity immunoassays		
IFN gamma Human ProQuantum™ Immunoassay Kit	96 tests	A35576
IL-8 Human ProQuantum™ Immunoassay Kit	96 tests	A35575
ProcartaPlex multiplex panels		
Immune Monitoring 65-Plex Human ProcartaPlex™ Panel	96 tests	EPX650-10065-901
Cytokine & Chemokine 36-Plex Mouse ProcartaPlex™ Panel 1A	96 tests	EPX360-26092-901