The new generation of target-specific protein quantitation

Introducing ProQuantum high-sensitivity immunoassays.

Many tools and strategies have been developed to advance our understanding of human diseases and their cellular mechanisms, as well as to design more effective screening, prevention, and treatment methods. However, a simple, scalable, and affordable process for validating biomarkers or measuring extremely low levels of analytes in limited, hard-to-obtain samples has seemed out of reach. Our latest platform innovation—Invitrogen™ ProQuantum™ high-sensitivity immunoassays—is a set of ready-to-use kits for quantifying low-abundance proteins in small sample volumes (as little as 2–5 µL of serum). These high-performance singleplex immunoassays run on any qPCR instrument and typically require 2 hours from sample to answer.

ProQuantum immunoassays: How they work

ProQuantum immunoassays work by leveraging proximity ligation assay (PLA™) technology. The combination of powerful proteomic tools, including high-specificity antibody–antigen binding and Invitrogen™ SiteClick™ antibody labeling, with genomic tools such as Applied Biosystems™ TaqMan® qPCR technology produces a detection method with both high sensitivity and a broad dynamic range (Figure 1). With a fast and easy workflow and very small sample consumption requirements, the ProQuantum immunoassay platform can detect much lower levels of protein analyte than traditional methods (Figure 2). Moreover, these immunoassays can quantify analytes over a concentration range of 5 orders of magnitude or more, minimizing the need for sample dilutions.

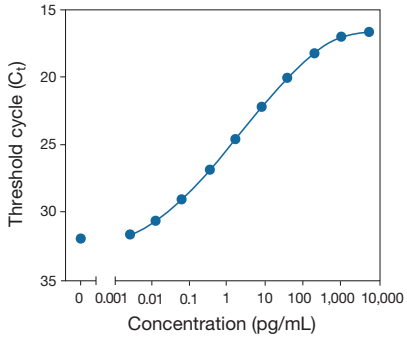

Figure 1. A 10-point standard curve for the ProQuantum human IL-8 immunoassay, demonstrating the assay's large dynamic range and sensitivity at extremely low target-protein concentrations. The IL-8 recombinant protein provided in the Invitrogen™ ProQuantum™ Human IL-8 Immunoassay Kit (Cat. No. A35575) was serially diluted 5-fold from a starting concentration of 5,000 pg/mL to 0.00256 pg/mL and then amplified according to the assay protocol to generate a standard curve that spans over 5 logarithmic units and shows low-end separation for quantitation of small amounts of protein. See Figure 3 for more details on the assay.

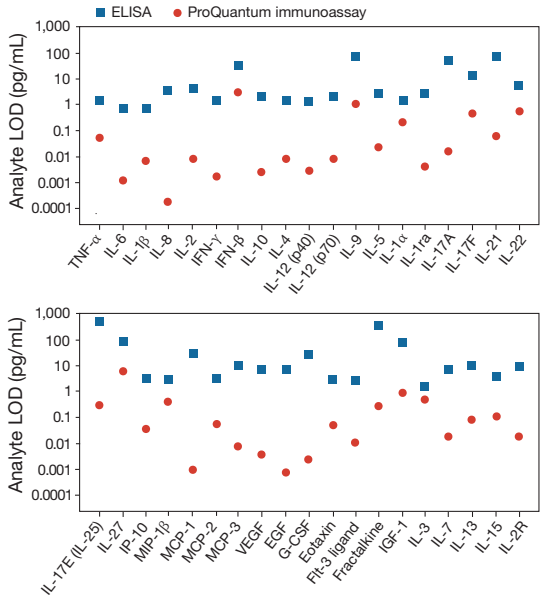

Figure 2. Analytical sensitivity comparison between ProQuantum and ELISA platforms. As compared with ELISAs, ProQuantum immunoassays show lower limit-of-detection (LOD) values for the same protein target. On average, ProQuantum immunoassays are 10- to 100-fold more sensitive than ELISAs.

The assay is based on target-specific antibodies that are methodically screened to develop an optimized pair of antibodies that bind to epitopes in close proximity on an antigen. These antibodies are preconjugated to DNA oligonucleotides at either the 3′ end (60 bases) or the 5′ end (40 bases) of the nucleic acid (Figure 3A). When the two antibodies are added to a sample suspension containing the specific protein to be quantitated, the two antibodies bind to their respective binding sites on the antigen, which results in the two oligonucleotide strands being brought in proximity (Figure 3A). This antibody binding provides structural stability such that in the presence of DNA ligase and a third oligonucleotide connector (complementary to the ends of each of the original 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 3B). Following a temperature increase to 95°C to inactivate the ligase and denature the analyte proteins and antibodies, the DNA templates are amplified through

40 cycles of TaqMan fluorescence-based qPCR. The amount of DNA produced is measured after each amplification cycle via the fluorescence increase, which is directly proportional to the number of PCR product molecules (or amplicons) generated.

Analysis of ProQuantum data

Figure 3 shows the ProQuantum immunoassay workflow and data produced. The fluorescence produced during amplification of the DNA template is measured throughout the qPCR cycling and plotted against the cycle number (Figure 3C). With high concentrations of protein analyte in the sample, a lower cycle number is needed to reach a given fluorescence threshold (threshold cycle or C_t); conversely, with lower protein concentrations, a higher cycle number is required. Analyte concentration values can be interpolated from a dose-response curve, generated using known protein concentrations (Figure 3D).

To facilitate quantitative measurements, we have developed intuitive, user-friendly, cloud-enabled software to provide easy standard curve fitting and analysis, including statistical groupwise comparison. The ProQuantum immunoassay is extremely sensitive, enabling quantitation of basal cytokine levels in normal human serum (Figure 4), as well as in other research model systems in which there is a need to interrogate low-abundance proteins.

A ProQuantum assay for your target

The ProQuantum immunoassay platform and software provide a simple yet sophisticated solution for quantitative immunoassays, with a typical sample-to-answer time of 2 hours. See the most up-to-date list of target proteins and learn more about ProQuantum immunoassays at thermofisher.com/proquantumbp76. ■

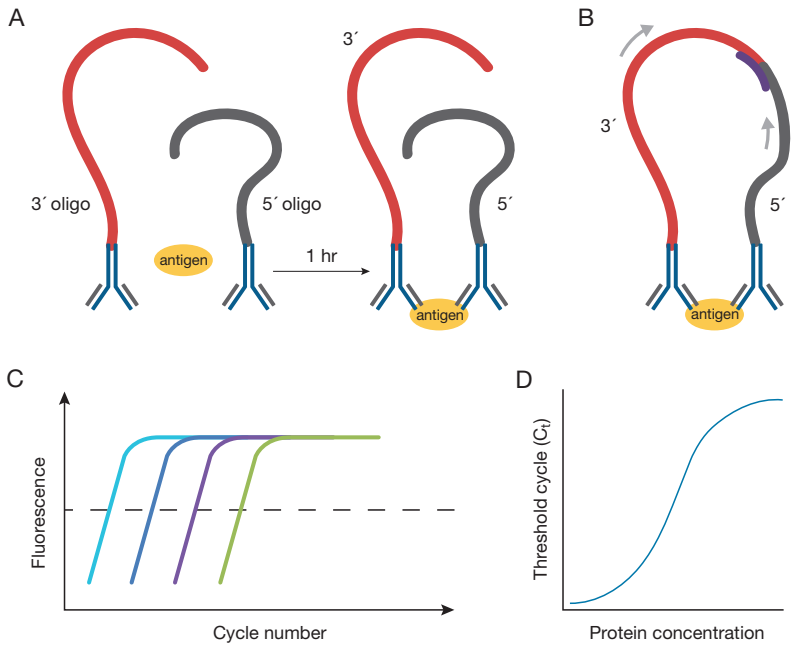

Figure 3. How ProQuantum immunoasssays work. (A) Antibody–antigen binding: Antibodies bind to two separate epitopes on the antigen (during a 1 hr incubation), which brings the two conjugated oligonucleotides into close proximity. **(B)** Ligation and amplification of signal (in a qPCR instrument): DNA ligase and a third oligonucleotide connector are added to ligate the two ends of the conjugated oligonucleotides, creating a 100-base DNA template. Once the ligase is inactivated at 95°C, the sample is amplified through 40 cycles of annealing and extension. **(C)** The amount of DNA produced is measured after each amplification cycle via fluorescent dyes, which exhibit fluorescence that is directly proportional to the number of PCR product molecules (or amplicons) generated. This graph shows the fluorescence vs. cycle number curves for four different starting protein 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. protein concentration to create a standard curve.

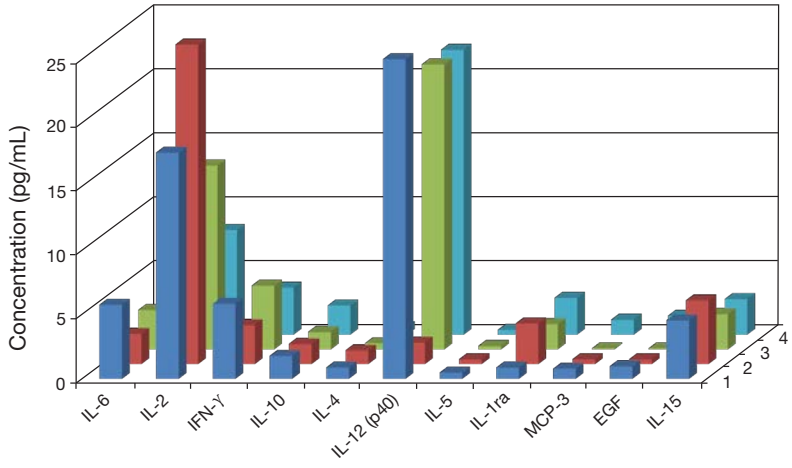

Figure 4. Sensitive cytokine detection in human serum using ProQuantum immunoassays. Sera from four healthy donors (labeled 1 through 4 on the z axis) were assayed with 11 different Invitrogen™ ProQuantum™ immunoassay kits to demonstrate the sensitivity of the assays. These low pg/mL concentrations were interpolated from standard curves generated for each target; none of the values fell outside of the lowest point on the standard curve.