Measure secreted protein and mRNA levels with a single instrument platform

ProcartaPlex and ProQuantum immunoassays augment mRNA quantitation.

Given the investigative tools available, biomedical researchers are sometimes constrained to choose either the study of genes (genomics) or the study of proteins (proteomics) to further the understanding of disease mechanisms and pursue the analysis of potential treatments, even though it is widely held that interrogation at both the gene and protein levels is valuable and necessary. By leveraging existing instrument platforms and lab expertise as well as crossover detection technologies, genomic and proteomic workflows can be combined without compromising data interpretation or sensitivity.

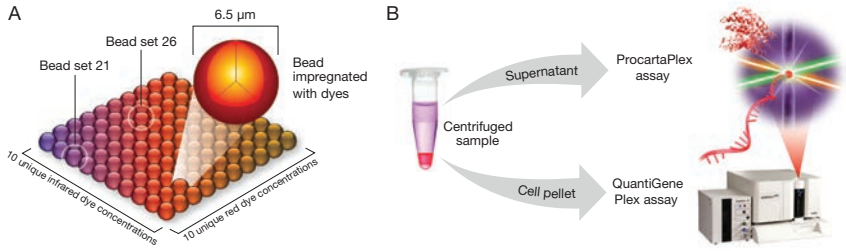

Figure 1. Levels of protein (ProcartaPlex assay) and gene expression (QuantiGene Plex assay) measured in a single sample using the Luminex® detection system.

Protein and mRNA detection with the Luminex detection system

One example of such a "dual-omic" platform is the Luminex® detection system and associated xMAP® magnetic bead technology, capable of multiplex analyte detection in a single microplate well (Figure 1). The Luminex® MagPlex® superparamagnetic microsphere beads are internally dyed with precise proportions of red and infrared fluorophores to create 100 spectrally unique signatures that can each be identified by the Luminex xMAP detection systems, including the Luminex® 200™, FLEXMAP 3D®, and MAGPIX® systems.

ProcartaPlex immunoassays. Predominantly adopted for protein quantitation—for example in the Invitrogen™ ProcartaPlex™ immunoassays—the xMAP bead technology allows the multiplex detection of up to 80 protein targets in a single microplate well, providing significant time and sample input savings over conventional ELISAs. ProcartaPlex immunoassays are antibody-based magnetic-bead reagent kits and panels for multiplex high-throughput quantitation of protein. Similar to a sandwich ELISA, the ProcartaPlex assay uses matched antibody pairs to identify the protein of interest; unlike conventional ELISAs, the capture antibody is conjugated to a free-floating magnetic bead and not adsorbed to the microplate well. In a ProcartaPlex multiplex assay, each spectrally unique bead is labeled with an antibody 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 distinct beads allows for analysis of multiple analytes in a single well. 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 available as preconfigured multiplex panels (2- to 65-plex) or singleplex kits that can be combined to create custom multiplex assays.

QuantiGene Plex mRNA assays. The xMAP technology has also been expanded and applied to the multiplex measurement of mRNA gene expression in the Invitrogen™ QuantiGene™ Plex Gene Expressions Assays, which are compatible with Luminex systems. QuantiGene Plex assays provide a method for multiplex high-throughput quantitation of mRNA, allowing the simultaneous measurement of up to 80 genes of interest in a single well of a 96- or 384-well plate using a Luminex xMAP detection system. With an ELISA-like workflow, the QuantiGene Plex assay begins with the direct hybridization of transcripts to magnetic beads, followed by signal amplification using branched DNA (bDNA) technology. In contrast with other gene expression assays, the QuantiGene Plex assay depends on signal amplification rather than target amplification for direct measurement of transcripts; i.e., no RNA purification or reverse transcription is required.

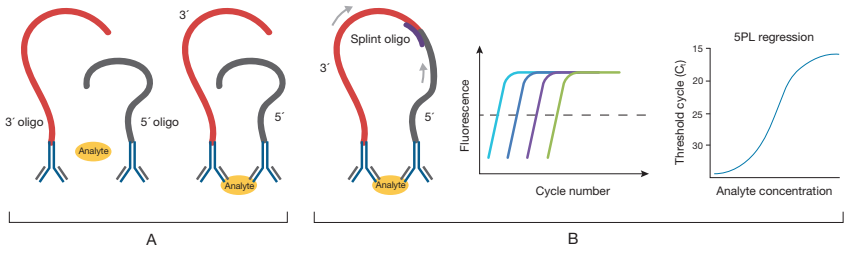

Figure 2. How ProQuantum immunoassays work. (A) Antibody conjugates bind to target during a 1 hr incubation. **(B)** With addition of a splint oligonucleotide and DNA ligase, the two antibody-conjugated oligonucleotides are ligated to create a 100-base template that is amplified through 40 qPCR cycles. The amount of DNA produced, measured via fluorescence, is directly proportional to the number of amplicons generated.

Protein quantitation using a qPCR platform: ProQuantum immunoassays

With qPCR reagents and instrumentation now so prevalent in research labs, Thermo Fisher Scientific has also recently launched a qPCR-based protein quantitation technology called the Invitrogen™ ProQuantum™ Immunoassay. ProQuantum high-sensitivity immunoassays provide an affordable and easily accessible protein quantitation method that can be used on the same instrument platform for mRNA quantitation.

Similar to ELISA kits, ProQuantum ready-to-use kits enable single-protein target detection and quantitation using matched antibody pairs. ProQuantum immunoassays, however, take advantage of Applied Biosystems™ TaqMan® fluorescence-based qPCR, providing both sensitivity and dynamic ranges that exceed those of conventional ELISAs (Figure 2). This immunoassay is based on an optimized pair of antibodies that bind to specific epitopes in close proximity on the analyte. These antibodies are preconjugated at the 3´ end of a 60-base oligonucleotide or the 5´ end of a 40-base oligonucleotide. When added to a sample containing the specific analyte, the two antibodies bind to their respective epitopes. In the presence of DNA ligase and a third splint oligonucleotide, the two antibody-conjugated oligonucleotides are ligated together to create a 100-base strand that can serve as a DNA amplification template. This template is then amplified through 40 qPCR cycles, and the amount of amplified product after each cycle, as measured by fluorescence, is directly proportional to the number of ligated templates created by the antibody–analyte binding. ProQuantum high-sensitivity immunoassays can quantify analytes over a concentration range of up to 5 orders of magnitude or more, minimizing the need for sample dilutions.

The ProQuantum immunoassay features a streamlined workflow with no wash steps. Moreover, only a total of 2 µL of serum is needed to obtain results in duplicate or triplicate, as compared with 50 µL volumes typically required for a single data point with an ELISA. The small sample-volume requirement means less work, not just during the assay run itself but also during sample collection procedures. Translational investigators working with finite human clinical samples must obtain as much data as they can from their often limited sample volumes. In addition, for small animal models like mice, longitudinal studies are often limited by the blood serum volumes required for each time point. With the small volume requirement of ProQuantum immunoassays, a simple tail prick is usually all that is required for each time point, and by using the same animal throughout the study, animal variability is also minimized.

Despite the small sample volumes, ProQuantum immunoassays can detect lower levels of proteins in samples than a traditional ELISA. For example, when comparing 40 endogenous natural sample measurements using either a mouse IL-1 alpha ELISA or a mouse IL-1 alpha ProQuantum immunoassay, only a single sample exhibited detectable levels of the IL-1 alpha protein using an ELISA, whereas 28 samples were quantifiable using the corresponding ProQuantum immunoassay (Figure 3). The assay range of the ProQuantum immunoassay spanned 0.32 to 5,000 pg/mL, whereas the ELISA provided an assay range of 8 to 500 pg/mL.

Explore these protein quantitation assays

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

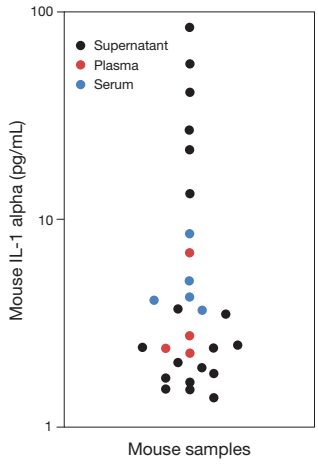

Figure 3. Use of a ProQuantum immunoassay with natural samples. Mouse IL-1 alpha levels were measured in natural samples (serum, plasma, and supernatant) using the Invitrogen™ IL-1 alpha Mouse ProQuantum™ Immunoassay Kit (Cat. No. A42894).