

invitrogen



ProQuantum high-sensitivity immunoassays

The next generation in protein quantitation

ThermoFisher
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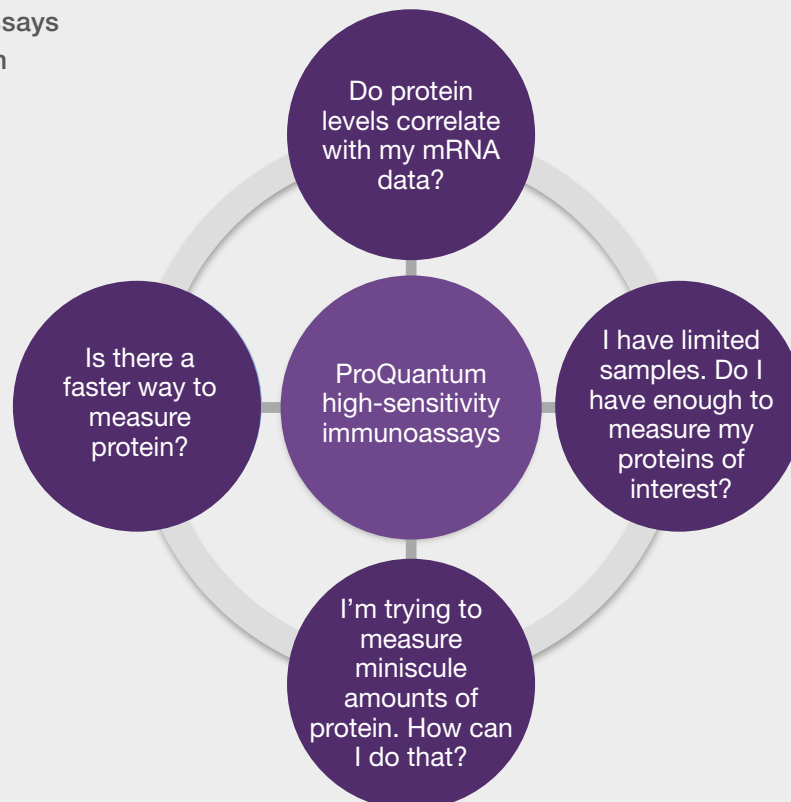
Measure protein analytes with high specificity and sensitivity

Invitrogen™ ProQuantum™ high-sensitivity immunoassays are a line of ready-to-use kits that provide an easy method of measuring protein analytes with high specificity and sensitivity using a very small amount of sample. The assay workflow is simple, with only one antibody incubation step and no washing prior to placing the plate in any qPCR instrument for signal detection. The data analysis is performed on newly designed, free cloud-based software. This is an affordable, scalable, open platform that provides easy accessibility for every researcher who wants to measure protein concentrations.

Benefits include:

- **High sensitivity**—detect low levels of proteins with greater sensitivity than other traditional methods like ELISA
- **Low sample consumption**—use 2–5 µL of sample (e.g., 2 µL vs. 75 µL for triplicate wells with other methods)
- **Fast, easy workflow**—no wash steps, typically 2 hours from sample to results
- **Broad dynamic range**—≥5 logarithmic units, minimizing the need to dilute samples to fall within the range
- **No expensive, proprietary instrument to purchase**—runs on any qPCR instrument
- **Includes intuitive, free cloud-based software**—for easy, robust data analysis and statistical groupwise comparison

ProQuantum immunoassays
can answer your protein
detection needs



How does it work?

ProQuantum immunoassays utilize proximity ligation amplification technology to combine antigen–antibody binding for analyte detection with qPCR signal amplification and readout (Figure 1).

The assay is a two-step process:

A. Analyte binding by paired antibodies conjugated to oligonucleotides

Two antibody conjugates are provided in each kit: a 3' end oligonucleotide and a 5' end oligonucleotide, each conjugated to a target-specific antibody. When the antibody pair binds to two different epitopes of the protein, the 3' and 5' oligos come into close proximity.

B. Ligation of the oligonucleotides by DNA ligase and amplification by Applied Biosystems™ TaqMan® qPCR Assay

Only when the pair of antibodies binds to the analyte (Figure 1A) can the associated oligos become bound to the complementary splint oligo and subsequently joined to each other with DNA ligase (Figure 1B). Following the oligo ligation, 95°C heat inactivation denatures the ligase, antibodies, and other proteins, leaving 100-base strands in concentrations proportional to the level of antibody–analyte binding in the first stage. This 100-base DNA strand serves as the amplification template for 40 cycles of qPCR using TaqMan Assays.

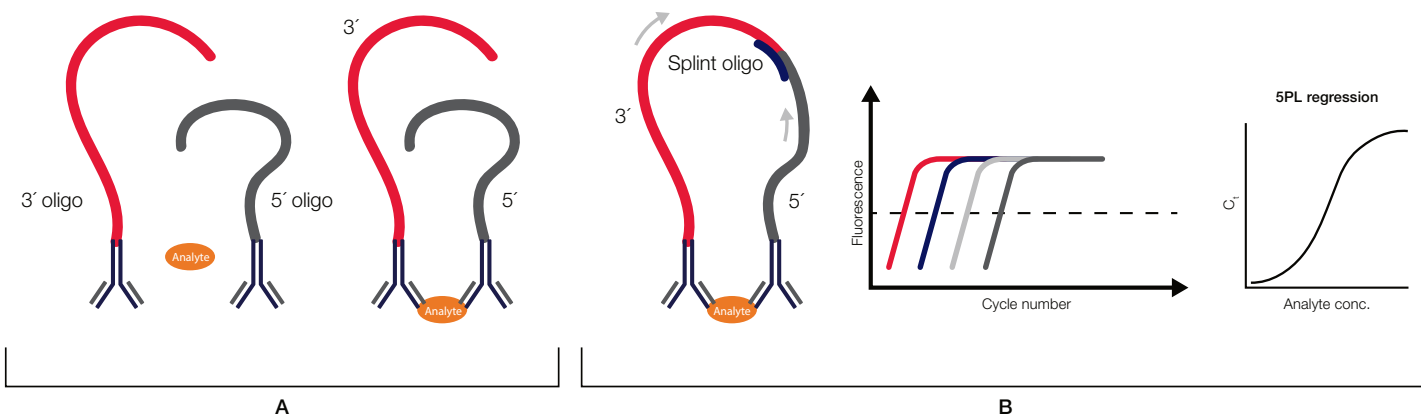


Figure 1. How ProQuantum immunoassays work.

What comes in each kit?

Each kit provides all of the reagents needed, in sufficient quantities to run 96 assay reactions (Figure 2).

Each kit contains:

- 2 protein standards (lyophilized)
- Assay dilution buffer
- Antibody-conjugate A
- Antibody-conjugate B
- Antibody-conjugate dilution buffer
- Master mix
- Ligase

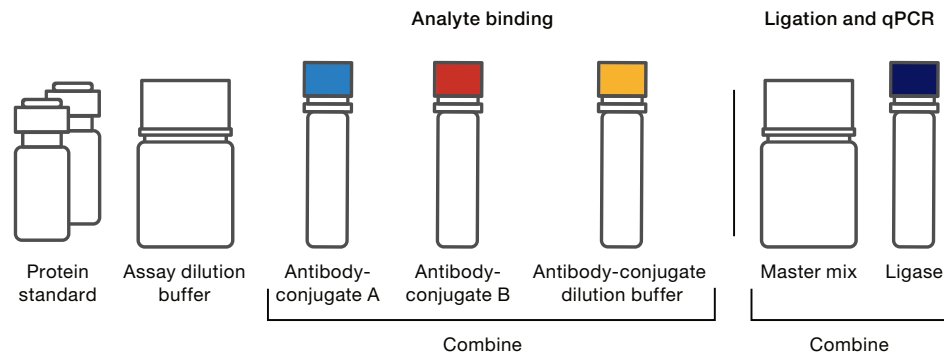


Figure 2. ProQuantum kit contents. Components are color-coded for ease of use.

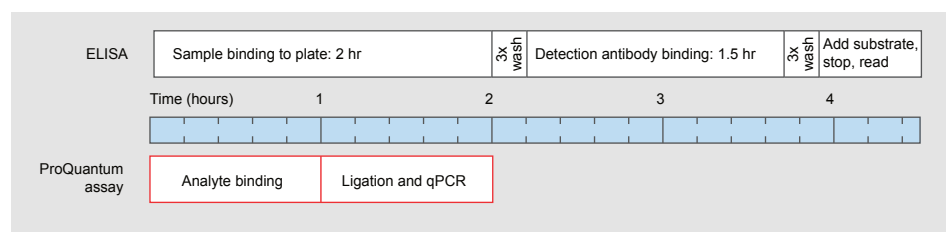


Figure 3. Assay run time compared to ELISA.

Assay workflow

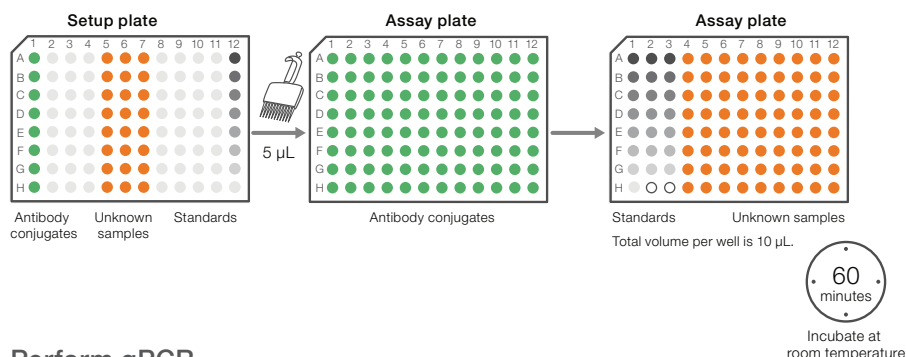
The assay workflow is simple. Compared to traditional methods like ELISA, there are fewer incubation steps and no wash steps, and time-to-results is faster (Figures 3 and 4).

A setup plate is recommended in order to do all the preparation steps, including mixing the antibody conjugates, 10-fold dilution of unknown samples, and serial dilutions of the protein standards.

The assay run is performed in 2 hours. The first incubation step allows the antibodies to bind to the target analyte for 1 hour. The next step is to add the qPCR reaction mixture and place the plate in the qPCR instrument for ligation and qPCR readout.

Bind analyte

Transfer 5 μ L of antibody-conjugate mixture to all wells. Then add 5 μ L of the standard or the unknown samples to appropriate wells.



Perform qPCR

Add 40 μ L of qPCR reaction mixture to all assay wells.

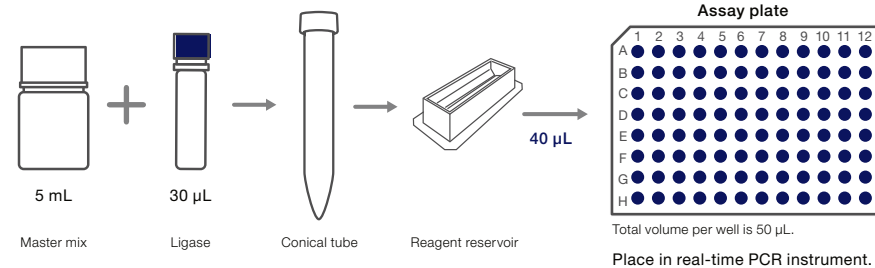


Figure 4. Schematic of assay run steps. This is only an example of running 5 μ L sample volumes in triplicate on an entire 96-well plate. Volumes can be scaled down to 2 μ L samples.

How has the assay been tested?

With over 32 years of immunoassay experience, we have developed a high-performance assay with the best quality testing in mind. The ProQuantum platform measures the target of interest with great sensitivity, dynamic range, and precision (Figures 5–8).

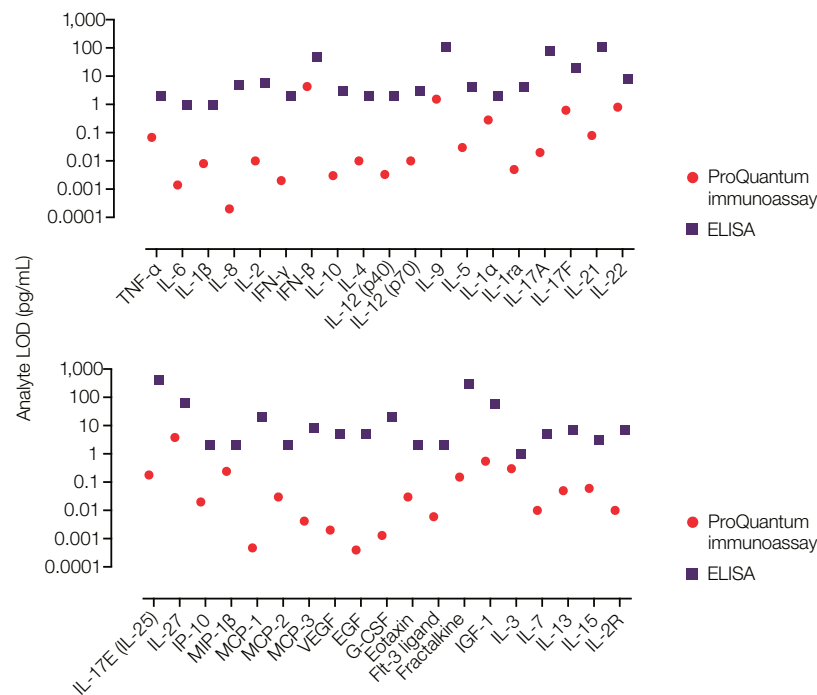


Figure 5. Analytical sensitivity. Limit of detection (LOD) is defined as 2 standard deviations above background. Comparison of LODs for a number of human cytokine targets demonstrates that the ProQuantum platform has 10–1,000x greater sensitivity than ELISA.

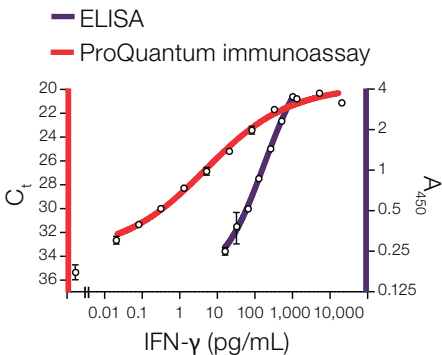


Figure 6. Broad dynamic range. The standard curve for the ProQuantum assay overlaid with that of an ELISA demonstrates the larger dynamic range as well as low-end separation.

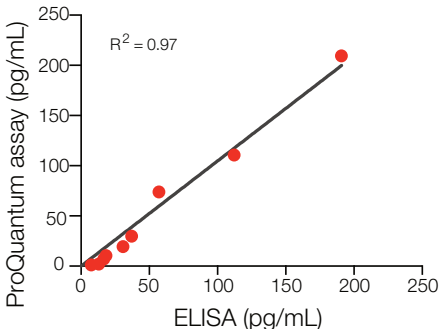


Figure 7. Correlation of human IL-8 ProQuantum vs. ELISA assays. Serum samples were tested by ELISA and show correlative data between the two platforms.

Parameters	Intra-assay precision			Inter-assay precision		
	Sample 1	Sample 2	Sample 3	Sample 1	Sample 2	Sample 3
Mean measured conc. (pg/mL)	439	91	21	409	88	20
CV (%)	6	6	8	1	1	6

Figure 8. Precision. Samples of known human IL-6 concentration were determined in replicates of 10 within an assay (intra-assay precision) or in 3 different assays (inter-assay precision).

Each kit has been tested on natural samples such as serum, and evaluated using tests such as expected protein levels, spike and recovery, linearity of dilution, and parallelism (Figures 9 and 10). The standards are calibrated to ELISAs, literature values, or NIBSC proteins, if available.

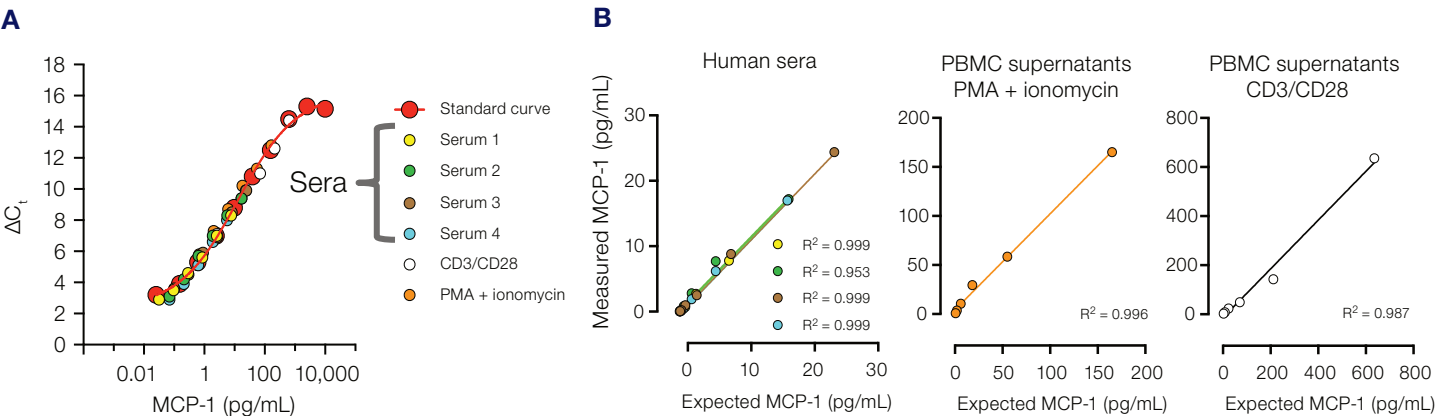


Figure 9. Parallelism and linearity of dilution. (A) Assays of a recombinant protein and the corresponding natural protein found in native samples show parallel results. Serum and supernatant samples were tested against the MCP-1 standard curve to show parallelism. (B) Linearity indicates that the observed signals in the standard curve match the general rate of dilution stoichiometry principles. Serum and supernatant samples show that the measured vs. expected results are the same in the linear range of the curve.

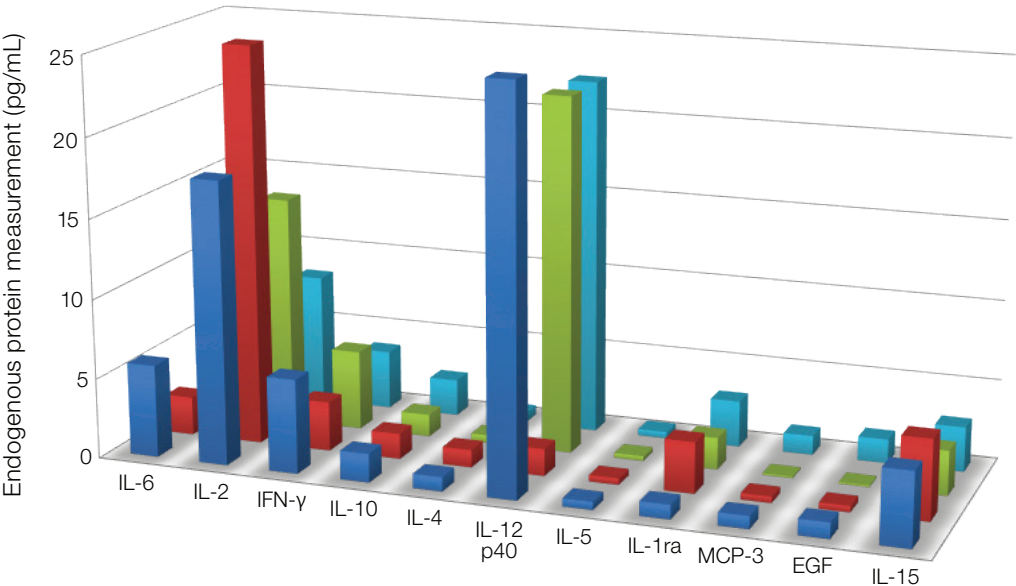


Figure 10. Detectable protein levels in normal human serum from 4 donors, measured using various ProQuantum immunoassays. All samples showed quantifiable protein levels within the range of their respective standard curves. These values often are not quantifiable on other platforms like ELISA.

Data analysis using cloud-based ProQuantum software

The intuitive Invitrogen™ ProQuantum™ cloud-based software is available to support your data analysis. This step-by-step software helps you set up your assay, including standard curve dilution calculation, plate layout design, and customized lab bench instructions (Figure 11). Once the assay has been performed at the lab bench, simply upload your run files from the qPCR instrument to the ProQuantum cloud app and analyze the data. Analysis features include standard curve qualification, outlier detection, 4 or 5PL regression fit, results for unknown samples in pg/mL, and groupwise statistics.

The modern laboratory is undergoing a technological revolution. Easily access and securely share results with colleagues anywhere, anytime, when using our Connect platform, part of the digital capabilities of Thermo Fisher Scientific. Access free ProQuantum cloud-enabled software at apps.thermofisher.com/apps/proquantum

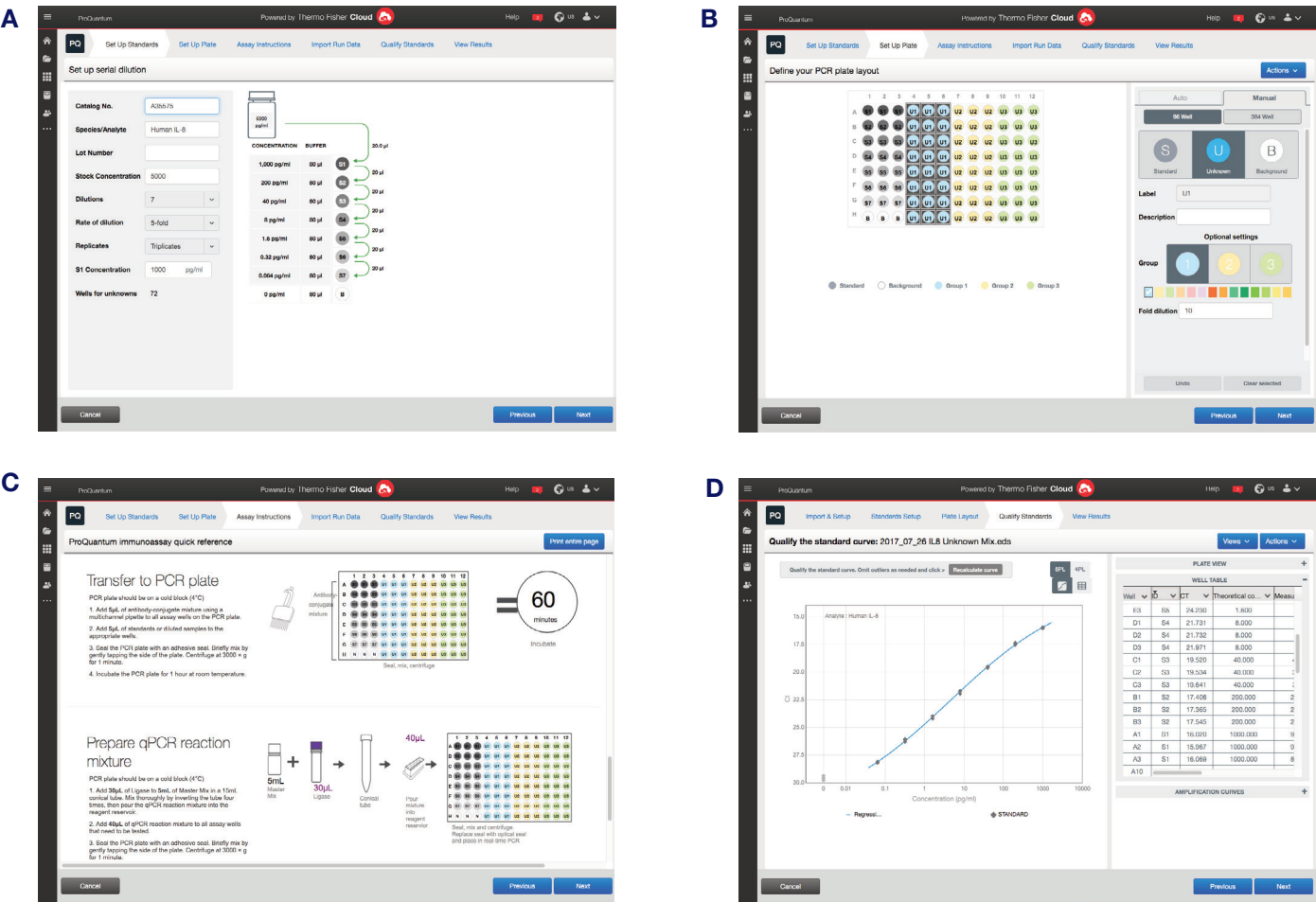


Figure 11. Assay setup and analysis using ProQuantum software. (A) Standard curve setup. (B) Plate setup. (C) Customized lab instructions. (D) Data and results.

Need a new qPCR instrument?

The Applied Biosystems™ QuantStudio™ 3 and 5 Real-Time PCR Systems provide our latest advancements in touchscreen usability, allowing you to stay connected to your data easily. They're designed for both new and experienced users who need simple and affordable real-time PCR systems without compromising on performance or quality. For more information and other system options, go to thermofisher.com/quantstudioqpcrfamily



* With internet access and our Connect platform.

New target-specific assays are being developed on a monthly basis. For any questions or to submit future protein target requests, contact us at invitrogenimmunoassays@thermofisher.com

For the most up-to-date product list and to learn more, go to thermofisher.com/proquantum

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