Olink offers an optimized, precise and efficient way to absolute quantification

Olink's novel method of absolute quantification allows users to skip time-consuming dilutions series, thereby minimizing the source of technical errors, increasing the calibration curve and data accuracy, as well as leaving more wells for samples.

Conventional immunoassays

Absolute quantification in immunoassays is commonly performed through generating a standard curve. This is accomplished through diluting a standard provided by the vendor into a series of known concentrations and is repeated for every plate that is run (Figure 1).

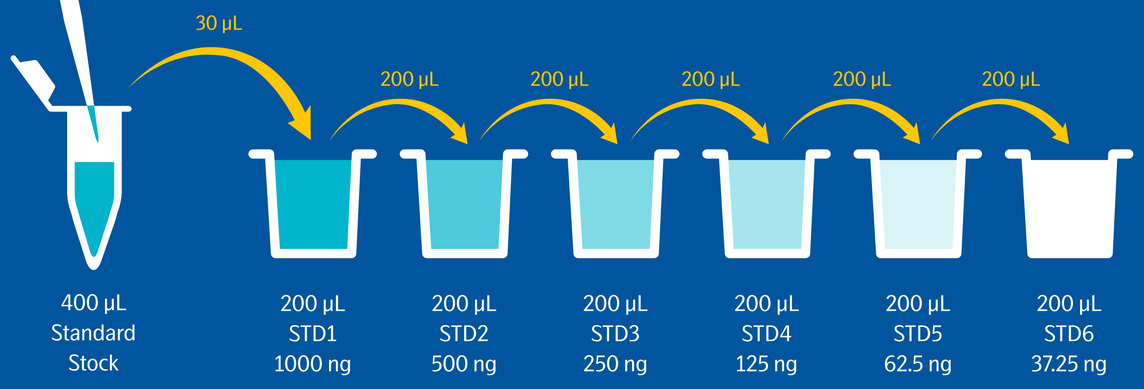

Figure 1: A standard dilution series is prepared for each experimental run.

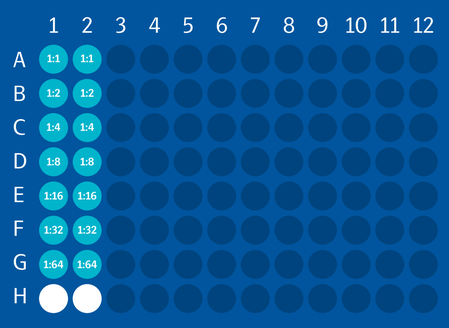

Figure 2: Standard dilutions are run in duplicates, along with negative controls.

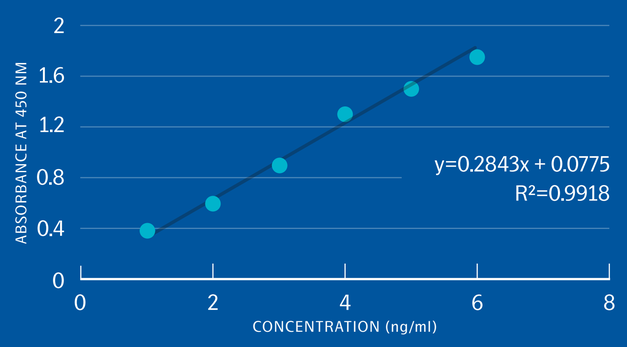

Figure 3: Unknown sample concentrations are then quantified based on the known quantity of the standard.

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Olink has developed a different way of performing absolute quantification using a calibrator system and pre-defined calibration curves.

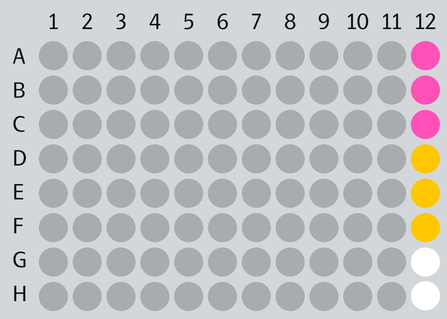

Figure 4:
- Calibrator
- Sample controls - pooled human plasma spiked with recombinant antigens
- Negative controls

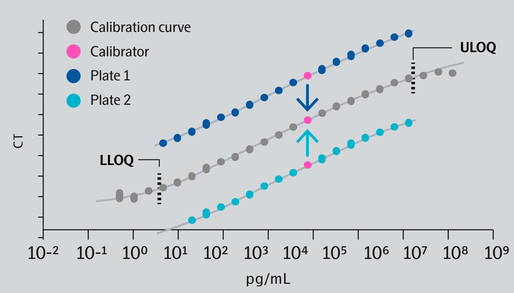

Figure 6: The Calibrator is run on each sample plate in triplicate and is used to adjust the predefined standard curves

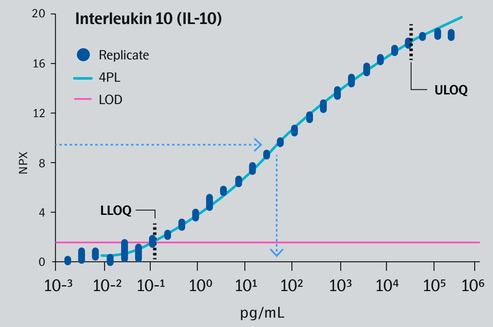

Figure 5: An example of a 24- point standard curve defined for each assay during development.

A 24-point standard curve with a 4PL curve fitting has been established for each protein during product development (Figure 5).

A single Calibrator point is measured in triplicates on each sample plate. For each run, the median value of this calibrator point is used to re-adjust the pre-defined calibration curves (Figure 6).

Measured sample values are related back to this adjusted standard curve model, translating the measured value to pg/ml.

The accuracy and precision of the calculated values are further validated by the known protein concentrations in the triplicate Sample Controls.

Disadvantages of the conventional absolute quantification:

- **The accuracy of pg/mL values is limited** due to the standard curve consisting of 6-8 points
- **Longer assay hands-on time and additional source of errors/inter-plate variation** by preparing the dilution series for each run
- **Lower throughput** with the dilution series run in duplicates, leaving fewer wells for samples, and using up more assay reagents

Benefits of Olink's absolute quantification method:

- **More precise pg/mL values** by higher resolution, 24-point calibrator curves (compared to standard 6-8 point)
- **Highest quality standard curves** by minimal pipetting errors and extremely accurate curve fitting, as a result of multiplex pipetting and running several replicates.
- **Less run variation** by pre-defined calibration curves that avoid operator dependent reconstitution and pipetting of standard curves.
- **Increased throughput** with less wells used for controls and standards

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