invitrogen

APPLICATION NOTE

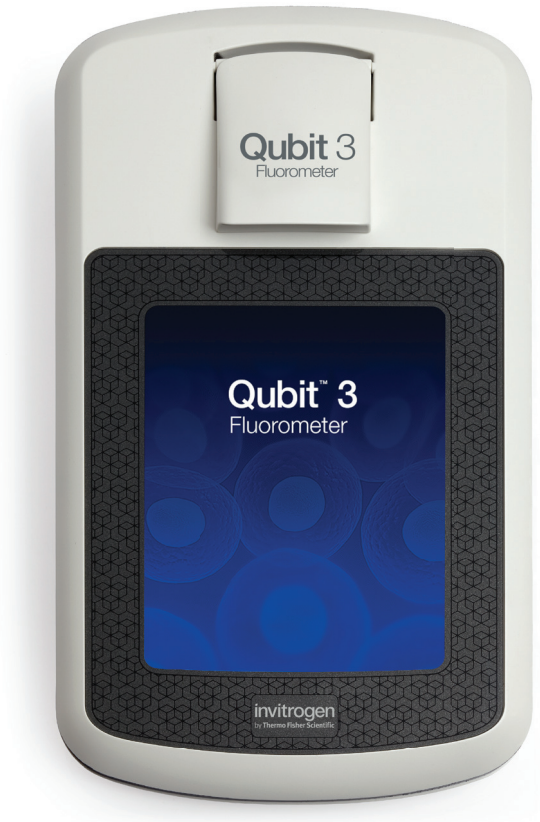
Qubit Fluorometer

Accurate and sensitive protein quantitation

Comparison of the Qubit Protein Assay for the Qubit Fluorometer and other conventional protein assays

Detection and quantitation of proteins are vital to many biological studies, due to the ubiquitous and fundamental roles of proteins in biological processes as well as the commercial importance of proteins in the biotech and pharmaceutical industries.

There are currently several absorbance- and fluorescence-based protein assays in common use, each with its own shortcomings, such as protein-to-protein variability, contaminant interference, time requirements, accuracy, sensitivity, and the need for caustic or hazardous reagents. We compared four common protein assays for protein-to-protein variation, accuracy, precision, and sensitivity. The Invitrogen™ Qubit™ Protein Assay for the Qubit™ Fluorometer compared favorably by providing low protein variability, rapid quantitation, accuracy, precision, and high sensitivity.



ThermoFisher SCIENTIFIC

The Qubit Quantitation Platform: fast and easy to use

The Invitrogen™ Qubit™ Quantitation Platform is the combination of a user-friendly fluorometer with highly sensitive fluorescence-based quantitation assays. The Qubit Fluorometer is a small, economical instrument designed to work seamlessly with Invitrogen Qubit Assay Kits for routine protein, DNA, and RNA quantitation (Figure 1). All settings and calculations are performed for you. The system is simple, fast, and easy to use, yet enables consistently accurate results, so you can be confident moving forward with subsequent applications. Each Qubit Assay Kit is highly specific for a single analyte, and all are more sensitive than absorbance-based measurements. Only small sample volumes of 1–20 µL are required, and all assay reagents are stored at room temperature, eliminating the need to thaw reagents.

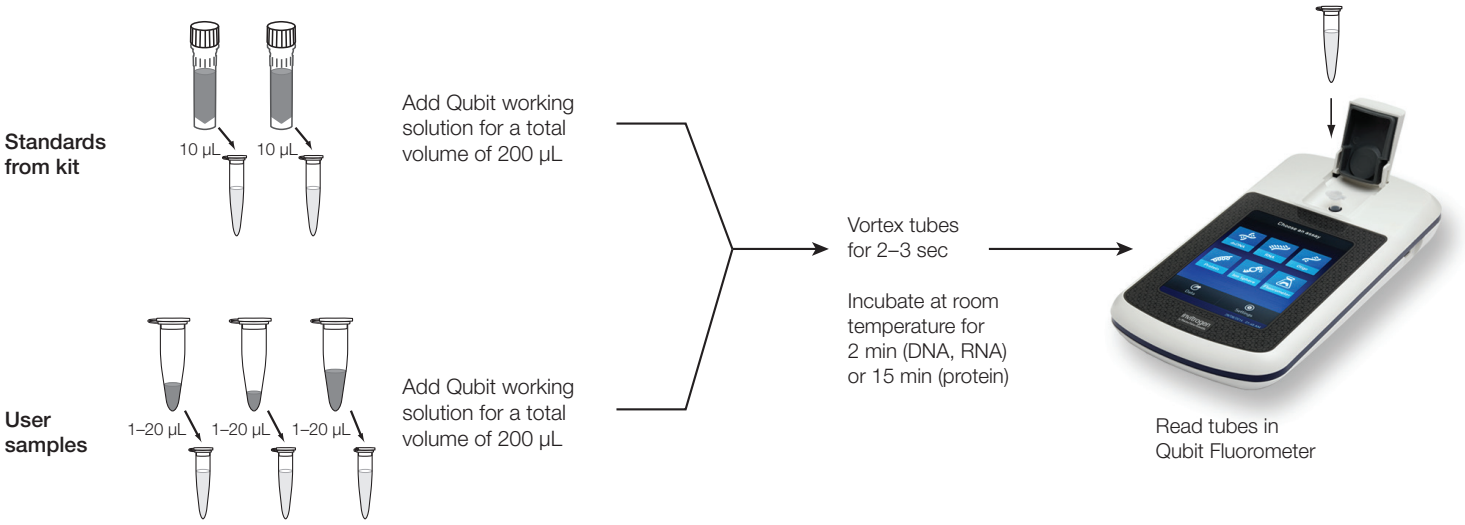


Figure 1. Workflow for the Qubit assays using the Qubit Fluorometer.

Qubit Protein Assay: accurate, precise, and sensitive

We compared 3 assays to the Qubit Protein Assay for the Qubit Fluorometer (Figure 2): the Bio-Rad™ Quick Start™ Bradford Protein Assay, the Pierce™ BCA Protein Assay, and the Pierce™ Modified Lowry Protein Assay. The Bradford method (using Coomassie Brilliant Blue) [1] exhibited very high protein-to-protein variability (Figure 2B). Spectrophotometric assays such as those using BCA (bicinchoninic acid) [2] require carefully timed steps, are not compatible with reducing agents, and can often yield high estimates of protein, as observed for lysozyme in our study (Figure 2C). The Lowry method [3] (Figure 2D) employs a lengthy, multistep procedure and is incompatible with detergents, carbohydrates, and reducing agents. The Qubit Protein Assay showed low protein-to-protein variation and good accuracy and precision, as well as sensitivity down to 0.025 mg/mL in the stock sample (Figure 2A).

The Qubit Protein Assay is insensitive to many common contaminants, including reducing agents, nucleic acids, and free amino acids. However, detergents such as SDS (final concentration >0.01%), Tween™ 20, and Triton™ X-100 are not recommended. The assay has an optimal range of 1.25 to 25 µg/mL (0.25–5 µg) in the assay tube (initial stock concentrations, 12.5–5 mg/mL) and is provided in a simple kit format that allows easy and rapid use.

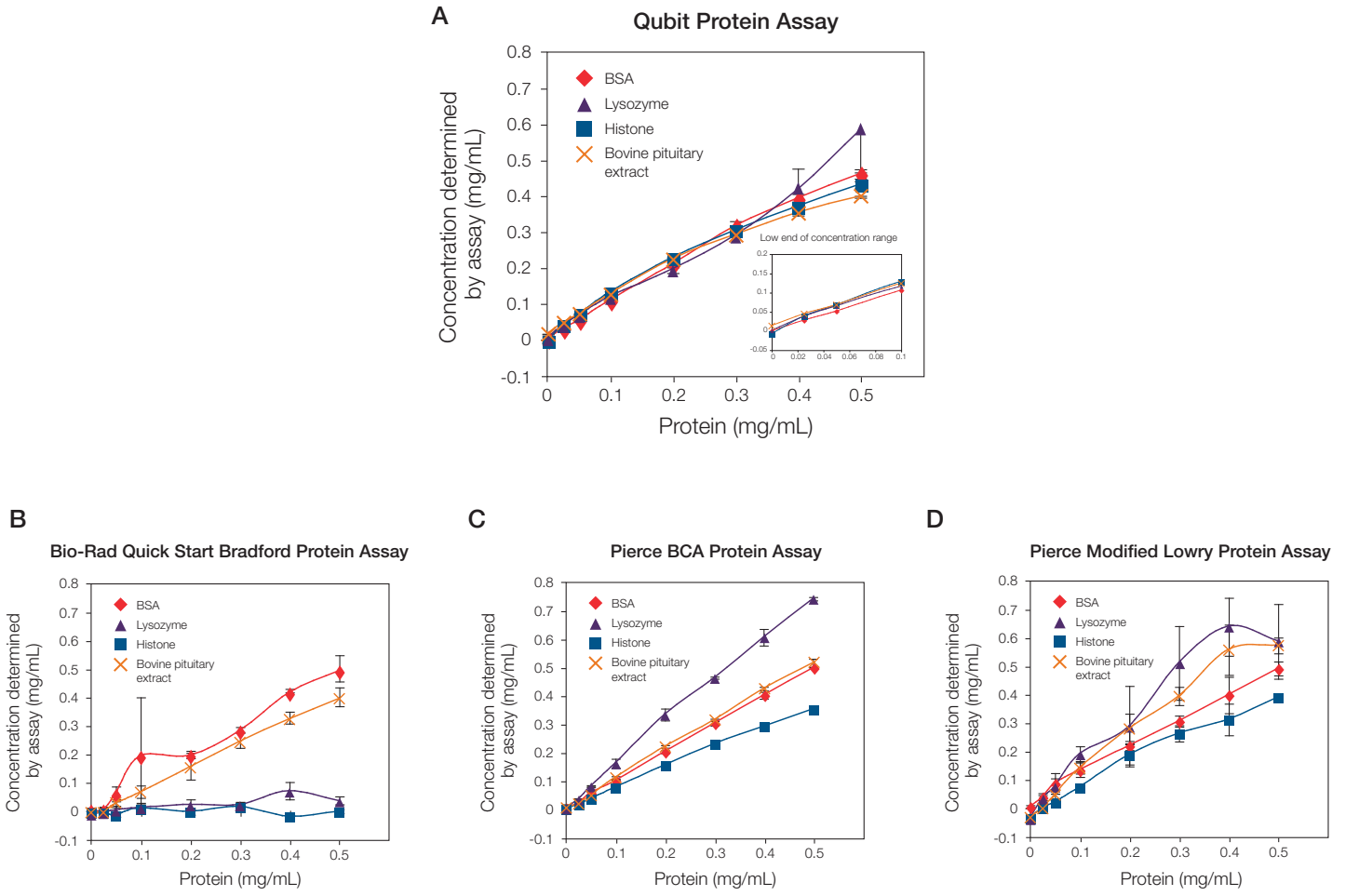


Figure 2. The Qubit Protein Assay with the Qubit Fluorometer produces less protein-to-protein variation and higher accuracy, precision, and sensitivity than three other common protein assays. (A–D) The same lot of each protein was used in all assays, and assays were carried out in triplicate following the manufacturers' protocols. BSA was the protein standard included in the Qubit Protein Assay Kit. Data are graphed to show protein-to-protein variation throughout the protein concentration range tested. The inset in (A) is a magnification of the low end of the protein range to show the sensitivity of the Qubit Protein Assay used with the Qubit Fluorometer.

invitrogen

Ordering information

Product	Quantity	Cat. No.
Qubit Protein Assay Kit, 0.25–5 µg	100 assays	Q33211
	500 assays	Q33212
Qubit RNA BR Assay Kit, 20–1,000 ng	100 assays	Q10210
	500 assays	Q10211
Qubit RNA HS Assay Kit, 5–100 ng	100 assays	Q32852
	500 assays	Q32855
Qubit ssDNA Assay Kit, 1–200 ng	100 assays	Q10212
Qubit dsDNA BR Assay Kit, 2–1,000 ng	100 assays	Q32850
	500 assays	Q32853
Qubit dsDNA HS Assay Kit, 0.2–100 ng	100 assays	Q32851
	500 assays	Q32854
Qubit 3 Quantitation Starter Kit	1 kit	Q33217
Qubit Assay Tubes	500 tubes	Q32856
Qubit 3 Fluorometer	1 each	Q33216

References

1. Bradford MM (1976) *Anal Biochem* 72:248–254.
2. Smith PK, Krohn RI, Hermanson GT et al. (1985) *Anal Biochem* 150:76–85.
3. Lowry OH, Rosebrough NJ, Farr AL et al. (1951) *J Biol Chem* 193:265–275.

Find out more at **thermofisher.com/qubit**

ThermoFisher SCIENTIFIC