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, because of 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, including the following:

- Protein-to-protein variability
- Contaminant interference
- Time requirements
- Accuracy
- Sensitivity
- Need for 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, providing low protein variability, rapid quantitation, accuracy, precision, and high sensitivity.

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Qubit Protein Assay: accurate, precise, and sensitive

We compared 3 assays to the Qubit Protein Assay for the Qubit Fluorometer (Figure 1): the Bio-Rad™ Quick Start™ Bradford Protein Assay, the Thermo Scientific™ 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 1B). 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 1C). The Lowry method [3] (Figure 1D) 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 sample (Figure 1A).

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 1.25–25 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.

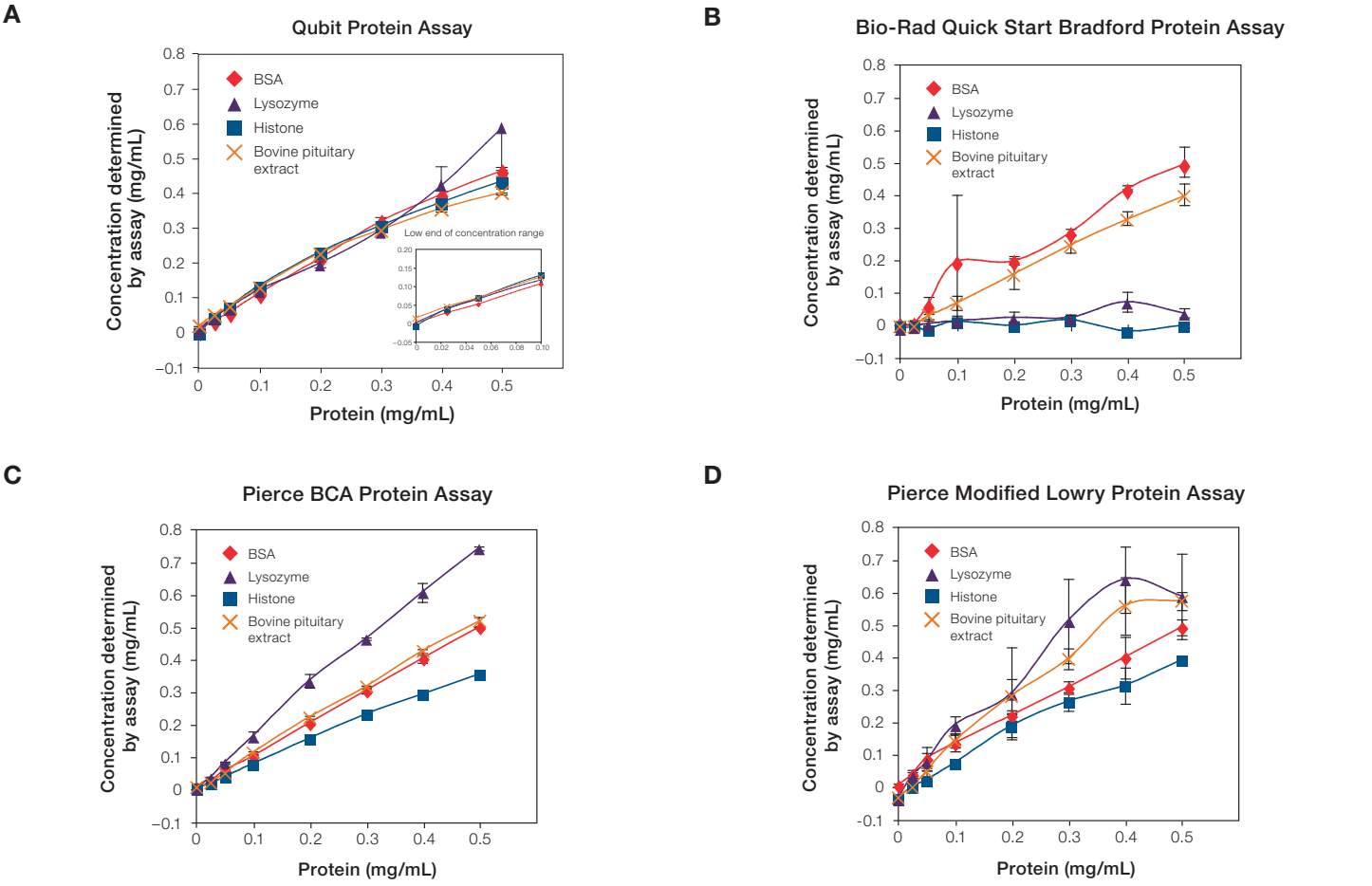

Figure 1. The Qubit Protein Assay with the Qubit Fluorometer produces less protein-to-protein variation and higher accuracy, precision, and sensitivity than three other common protein assays. (A–D) The same lot of each protein was used in all assays, and assays were carried out in triplicate following the manufacturers' protocols. BSA is 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.

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 2). 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. Only small sample volumes of 1–20 µL are required.

Key features of the Qubit 4 Fluorometer include:

- Qubit assay dyes bind selectively to DNA, RNA, or protein, making them more sensitive than UV absorbance
- Assays use as little as 1 µL of sample, even with very dilute samples
- Fast, reliable detection of degraded RNA with the new Invitrogen™ Qubit™ RNA IQ Assay
- New integrated reagent calculator to quickly generate working solution calculations

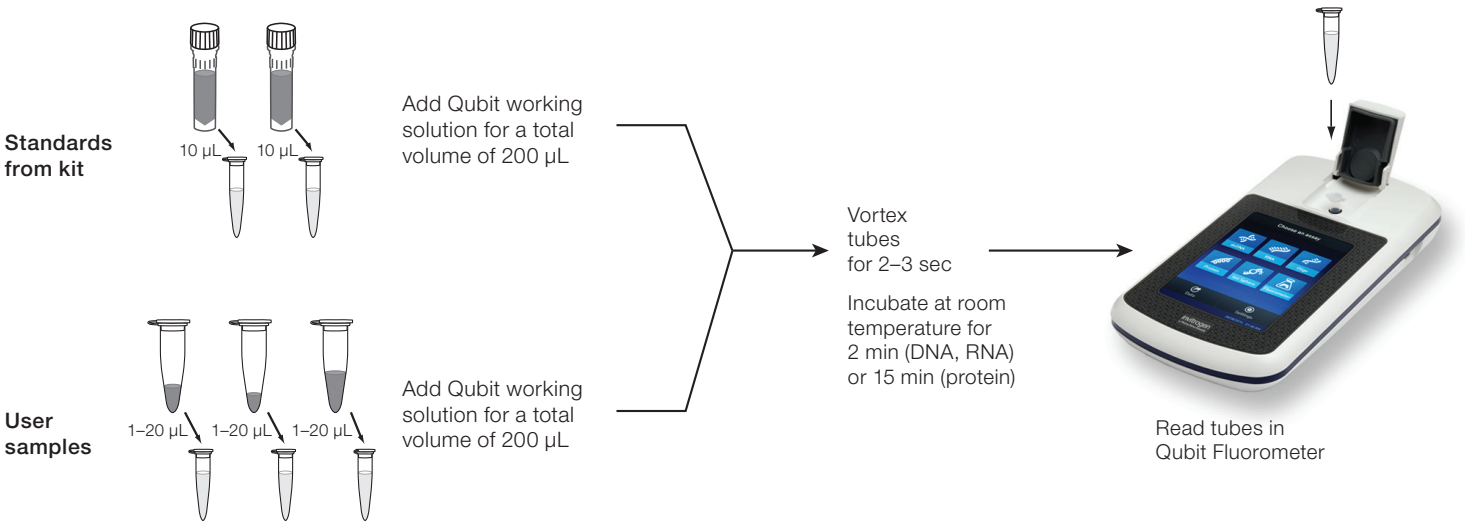

Figure 2. Workflow for the Qubit assays using the Qubit Fluorometer.

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Ordering information

Product	Initial sample concentration	Quantitation range	Quantity	Cat. No.
Protein kit				
Qubit Protein Assay Kit	12.5 µg/mL to 5 mg/mL	0.25–5 µg	100 assays	Q33211
			500 assays	Q33212
DNA kits				
Qubit ssDNA Assay Kit	50 pg/µL to 200 ng/µL	1–200 ng	100 assays	Q10212
Qubit dsDNA BR Assay Kit	100 pg/µL to 1,000 ng/µL	2–1,000 ng	100 assays	Q32850
			500 assays	Q32853
Qubit dsDNA HS Assay Kit	10 pg/µL to 100 ng/µL	0.2–100 ng	100 assays	Q32851
			500 assays	Q32854
Qubit 1X dsDNA HS Assay Kit	10 pg/µL to 100 ng/µL	0.2–100 ng	100 assays	Q33230
			500 assays	Q33231
RNA kits				
Qubit RNA BR Assay Kit	1 ng/µL to 1,000 ng/µL	20–1,000 ng	100 assays	Q10210
			500 assays	Q10211
Qubit RNA HS Assay Kit	250 pg/µL to 100 ng/µL	5–100 ng	100 assays	Q32852
			500 assays	Q32855
Qubit RNA XR Assay Kit	1 ng/µL to 8 µg/µL	20 ng–8 µg	100 assays	Q33223
			500 assays	Q33224
Qubit microRNA Assay Kit	50 ng/mL to 100 µg/mL	1–100 ng	100 assays	Q32880
			500 assays	Q32881
Qubit RNA IQ Assay Kit	NA	NA	75 assays	Q33221
			275 assays	Q33222
Instrument and accessories				
Qubit 4 Fluorometer			1	Q33226
Qubit 4 Quantitation Starter Kit			1 kit	Q33227
Qubit 4 NGS Starter Kit			1 kit	Q33228
Qubit 4 RNA IQ Starter Kit			1 kit	Q33229
Qubit Assay Tubes			500 tubes	Q32856

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.

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