Qubit Protein BR Assay—fast, accurate protein quantitation

Protein quantitation is an integral part of many protein biology workflows and a necessary step before commonly used techniques such as protein electrophoresis, western blotting, mass spectrometry, and immunoassays. The Invitrogen™ Qubit™ Protein BR Assay is a fluorometric assay that combines accuracy, compatibility, and ease of use, making protein concentration determination easier and faster.

The Qubit Protein BR Assay is optimized to work with a wide range of sample concentrations and components.

The assay is easy to perform and only requires a 10 minute incubation at room temperature (RT), eliminating the need to wait for long incubation periods or expose samples to elevated temperatures. The assay protocol, seen in Figure 1, is easy to set up with just two standards to prepare, unlike traditional assays that typically require a 7-point standard curve for quantitation.

Here we demonstrate the utility of the Qubit Protein BR Assay and compare it with many well-known assays used for protein quantitation.

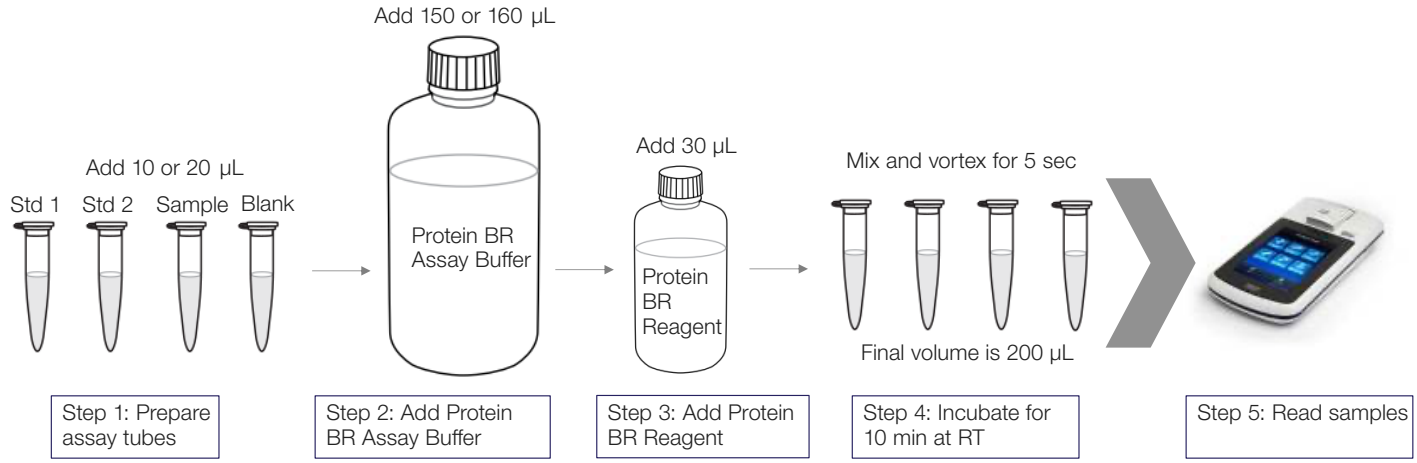

Figure 1. Qubit Protein BR Assay protocol.

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Key features

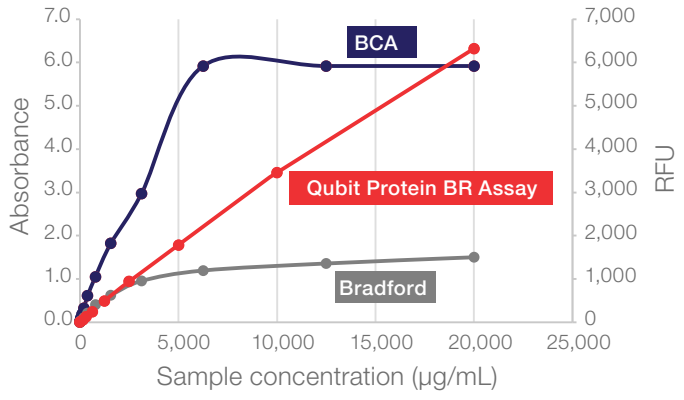
- Rapid assay with only 2 standards to prepare and 10 min incubation
- Compatible with detergents and reducing agents
- Broad dynamic range, 100–20,000 µg/mL

Broad dynamic range

One of the major advantages of using the Qubit Protein BR Assay is its broad dynamic range in comparison to standard colorimetric protein assays. The broad linear response allows accurate determination of unknown protein concentrations and provides a higher dynamic range than other standard protein assays (Figure 2). The Qubit Protein BR Assay can be used to detect protein concentrations from 100 to 20,000 µg/mL, allowing most samples to be used neat (undiluted), eliminating the guesswork and dilution steps that accompany standard protein quantitation methods.

Accurate protein determination

The Qubit Protein BR Assay provides accurate protein quantitation with low protein-to-protein variability as compared to traditional assays such as the Bradford assay. Proteins are diverse in their composition and structure, and differences in amino acid sequence, isoelectric point (pI), secondary structure, and side chains or prosthetic groups can result in variation in the quantitated concentration.



	Working range
Qubit Protein BR Assay	100–20,000 µg/mL
BCA Assay	20–2,000 µg/mL
Bradford Assay	125–1,500 µg/mL

Figure 2. Standard curves for protein quantitation assays. Purified bovine serum albumin (BSA) in 0.9% saline (0–20 mg/mL) was used to generate standard curves for the Qubit Protein BR Assay (red), Thermo Scientific™ Pierce™ BCA Protein Assay (blue), and the Bradford assay (gray). Assays were conducted following the manufacturers' protocols. The BCA and Bradford assays were performed in microplate format.

To demonstrate the accuracy and low protein-to-protein variability of the Qubit Protein BR Assay, several different cell lysates were generated, and total protein concentration was determined with the Qubit Protein BR Assay and a Bradford protein assay. Based on the calculated concentrations, the amount of each lysate containing 10 µg of protein was loaded onto a protein gel. The accuracy of the total protein loads was evaluated using Invitrogen™ No-Stain™ Protein Labeling Reagent in combination with lane normalization analysis on an Invitrogen™ iBright™ FL1500 Imaging System. The load variation produced by the Qubit Protein BR Assay was relatively low, with a coefficient of variation (CV) of 11%, whereas the load variation produced by the Bradford assay was 2.5 times higher, with a CV of 28% (Figure 3).

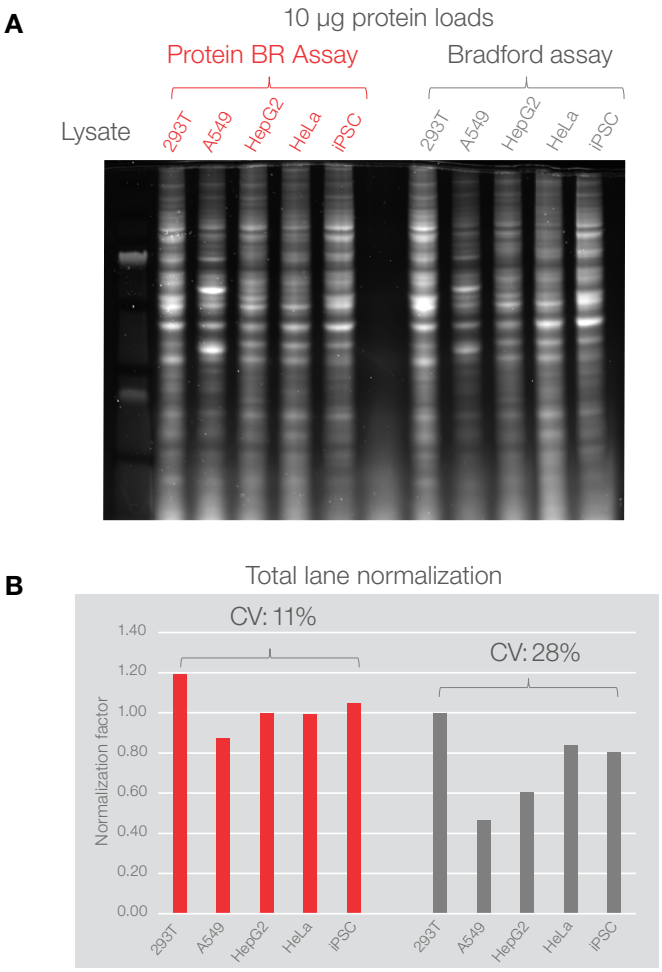


Figure 3. Accurate determination of protein load from complex protein mixtures. The Qubit Protein BR Assay and a standard Bradford assay were used to determine the protein concentration of lysates from several mammalian cell types: 293T, A549, HepG2, HeLa, and iPSCs. Lysates were separated on an Invitrogen™ NuPAGE™ 4–12% Bis-Tris Mini Protein Gel and labeled with No-Stain Protein Labeling Reagent. **(A)** Gel image was acquired on the iBright FL1500 Imaging System, and **(B)** normalization factors were determined using the Invitrogen™ iBright™ Analysis Software.

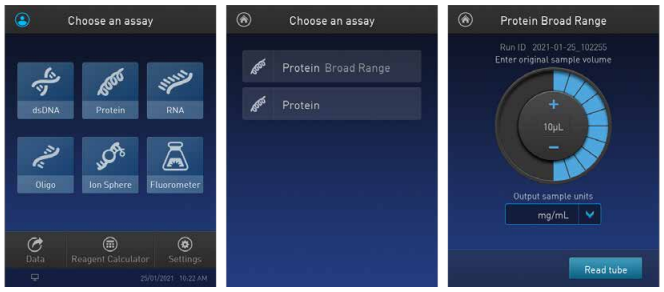
Reagent compatibility

The Qubit Protein BR Assay has a unique advantage over other standard protein quantitation assays such as the BCA and Bradford assays—it is compatible with samples that contain up to 5% detergent and compatible with commonly used reducing agents. The Qubit Protein BR Assay can be performed with many of the commonly used buffers and tolerates contaminants found in typical protein analysis buffers. Table 1 presents a summary of the substances tested and their compatible concentrations. Table 2 provides the formulations of the buffers tested.

Qubit quantitation platform—fast and easy to use

The Qubit Protein BR Assay is optimized for the Invitrogen™ Qubit™ 4 Fluorometer. The Qubit 4 Fluorometer provides the combination of a user-friendly fluorometer and capability to run highly sensitive fluorescence-based quantitation assays. The Qubit 4 Fluorometer is a small, economical instrument designed to work seamlessly with Invitrogen™ Qubit™ assay kits for routine protein, DNA, and RNA quantitation. All settings and calculations are performed directly on the instrument. The system is simple, fast, and easy to use, yet enables consistently accurate results for subsequent applications. Only small sample volumes of 1–20 µL are required for all assays.

Assay setup



Results

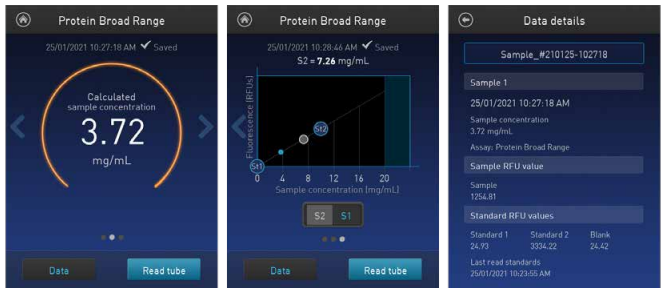


Figure 4. User interface for the Qubit Protein BR Assay on the Qubit 4 Fluorometer.

Table 1. Assay compatibility with common buffer components.

Contaminant	Concentration in sample buffer
β-Mercaptoethanol	1 mM
Acetonitrile	20%
Ammonium sulfate	200 mM
Bicine	100 mM
Borate (50 mM), pH 8.5	Undiluted
B-PER Reagent	Undiluted
CHAPS	5%
Carbonate-bicarbonate	Undiluted
Dithiothreitol (DTT)	5 mM
DMSO	10%
EDTA	50 mM
Glucose	1 M
Glycerol	10%
Guanidine-HCl	4 M
Imidazole	200 mM
I-PER Reagent	Undiluted
Mem-PER Protein Extraction Reagent	Undiluted
MES	125 mM
MOPS	100 mM
M-PER Reagent	Undiluted
NE-PER (CER) Reagent	Undiluted
NE-PER (NER) Reagent	Undiluted
NP-40	5%
Phosphate-buffered saline (PBS), pH 7.4	Undiluted
PMSF	1 mM
RIPA	Undiluted
SDS	5%
Sodium acetate	100 mM
Sodium chloride	5 M
Sucrose	20%
T-PER Tissue Protein Extraction Reagent	Undiluted
Tricine	50 mM
Tris-buffer saline (TBS)	Undiluted
Tris-glycine, pH 8.0	Ø*
Tris-glycine SDS, pH 8.3	Ø*
Tris-HCl	500 mM
Tris-HEPES SDS, pH 8.0	Undiluted
Triton X-100	5%
Tween 20	3%
Urea	3 M
Y-PER Yeast Protein Extraction Reagent	Ø*
Pierce GPCR Extraction and Stabilization Reagent	50%
Pierce Cell Surface Protein Isolation Kit	Undiluted

* Ø denotes incompatibility at the lowest concentration tested.

Table 2. Buffer formulations used in compatibility testing.

Buffer	Formulation
Sodium carbonate-bicarbonate	0.2 M sodium carbonate-bicarbonate, pH 9.4
PBS	100 mM sodium phosphate, 150 mM NaCl, pH 7.2
RIPA buffer	25 mM Tris, 150 mM NaCl, 1% DOC, 1% NP-40, 0.1% SDS, pH 7.6
TBS	25 mM Tris, 150 mM NaCl, pH 7.4
Tris-glycine	25 mM Tris, 192 mM glycine, pH 8.0
Tris-glycine-SDS	25 mM Tris, 192 mM glycine, 0.1% SDS, pH 8.3
Tris-HEPES-SDS	100 mM Tris, 100 mM HEPES, 3 mM SDS

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Methods

Qubit Protein BR Assay

For each standard or sample, 20 µL was dispensed in replicate into 0.5 mL thin-walled PCR tubes. To each assay tube, 150 µL of Qubit Protein BR Assay Buffer was added, followed by the addition of 30 µL of Qubit Protein BR Assay Reagent. The assay tubes were immediately vortexed for 5–7 sec after the addition of the Qubit Protein BR Assay Reagent and incubated at RT for 10 min. Assay tubes were read on the Qubit 4 Fluorometer.

Gel loading accuracy

Lysates from 293T, A549, HepG2, HeLa, and iPSC mammalian cell lines were grown to 80% confluency. Cells were lysed with Thermo Scientific™ M-PER™ Mammalian Protein Extraction Reagent containing Thermo Scientific™ Halt™ Protease Inhibitor Cocktail (Cat. No. 78439). The protein concentration of each lysate was determined using the Qubit Protein BR Assay and a standard Bradford assay. The Qubit Protein BR Assay was performed as described above. The Bradford assay was performed according to the manufacturer's instructions in a microplate format. An amount of each lysate containing 10 µg of protein was separated on an Invitrogen™ NuPAGE™ Bis-Tris 4–12% gel (Cat. No. NP0321BOX) following the protein assays. The gel was labeled with No-Stain Protein Labeling Reagent (Cat. No. A44449) according to the manufacturer's instructions. The labeled gel was imaged on an iBright FL1500 Imaging System, and data were analyzed using cloud-based iBright Imaging Analysis Software.

Reagent compatibility

The Qubit Protein BR Assay was performed as described above with samples of 1,000 µg/mL of BSA containing commonly used buffers and contaminants. Assays were performed in triplicate, and RFU values were compared to those of BSA in 0.9% saline, 0.05% sodium azide. The assay was considered compatible with the tested substance at the indicated concentration if there was less than 10% error in the protein concentration estimation in the presence of the substance.

Ordering information

Product	Initial sample concentration	Quantitation range	Quantity	Cat. No.
Protein kits				
Qubit Protein BR Assay Kit	100 µg/mL–20 mg/mL	1–400 µg	100 assays	A50668
			500 assays	A50669
Qubit Protein Assay Kit	12.5 µg/mL–5 mg/mL	0.25–5 µg	100 assays	Q33211
			500 assays	Q33212
DNA kits				
Qubit ssDNA Assay Kit	50 pg/µL–200 ng/µL	1–200 ng	100 assays	Q10212
Qubit dsDNA BR Assay Kit	100 pg/µL–1,000 ng/µL	2–1,000 ng	100 assays	Q32850
			500 assays	Q32853
Qubit dsDNA HS Assay Kit	10 pg/µL–100 ng/µL	0.2–100 ng	100 assays	Q32851
			500 assays	Q32854
Qubit 1X dsDNA BR Assay Kit	200 pg/µL–4,000 ng/µL	4–4,000 ng	100 assays	Q33265
			500 assays	Q33266
Qubit 1X dsDNA HS Assay Kit	10 pg/µL–100 ng/µL	0.2–100 ng	100 assays	Q33230
			500 assays	Q33231
RNA kits				
Qubit RNA BR Assay Kit	1 ng/µL–1,000 ng/µL	20–1,000 ng	100 assays	Q10210
			500 assays	Q10211
Qubit RNA HS Assay Kit	250 pg/µL–100 ng/µL	5–100 ng	100 assays	Q32852
			500 assays	Q32855
Qubit RNA XR Assay Kit	1 ng/µL–8 µg/µL	20 ng–8 µg	100 assays	Q33223
			500 assays	Q33224
Qubit microRNA Assay Kit	50 pg/µL–100 ng/µL	1–100 ng	100 assays	Q32880
			500 assays	Q32881
Qubit RNA IQ Assay Kit	NA	NA	75 assays	Q33221
			275 assays	Q33222
Instruments and accessories				
Qubit 4 Fluorometer with Wi-Fi			1 instrument	Q33238
Qubit 4 Protein BR Starter Kit			1 kit	A51292
Qubit Assay Tubes			500 tubes	Q32856
Qubit 4 Quantitation Starter Kit, with Wi-Fi			1 kit	Q33239

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