Qubit™ Protein BR Assay Kit

Catalog Numbers A50668 and A50669

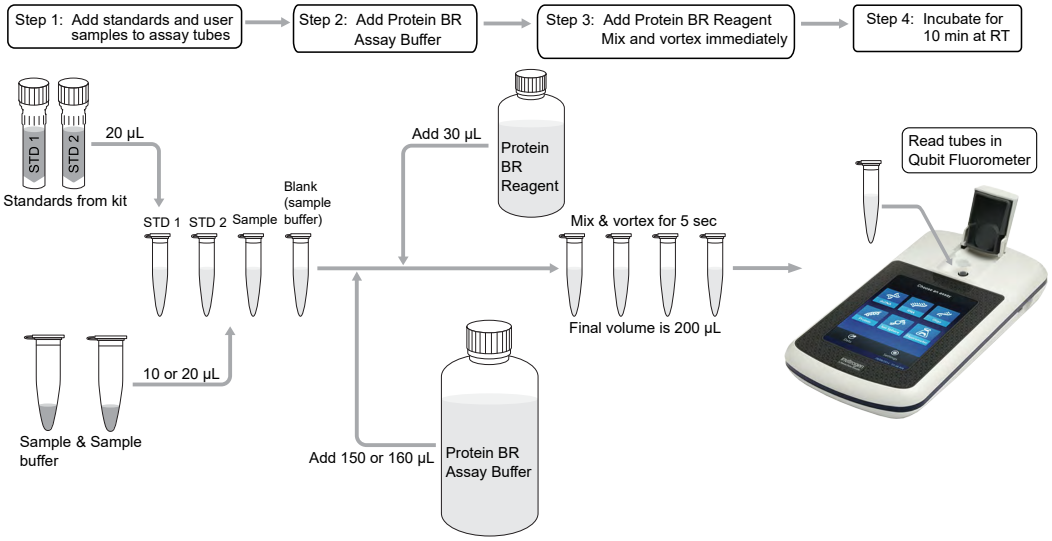
Pub. No. MAN0024790 Rev. B.0

WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from thermofisher.com/support.

Product description

The Invitrogen™ Qubit™ Protein BR Assay Kit provides a quick and accurate method to quantitate protein samples over a broad range of concentrations on the Qubit™ 4 Fluorometer. The kit includes Protein BR Assay Reagent, Protein BR Assay Buffer, Standard 1 (0 mg/mL BSA) and Standard 2 (10 mg/mL BSA). The assay is accurate for sample concentrations from 100 µg/mL to 20 mg/mL. The assay is performed at room temperature in an easy mix-and-read format that may be read within 10 minutes and up to 1 hour after addition. The assay is compatible with common contaminants found in protein preparations (see Table 1). For test samples containing low protein concentrations (<100 µg/mL), we recommend the Qubit™ Protein Assay Kit (Cat. No. Q33211).

The Qubit™ Protein BR Assay is based on the reaction between the labeling reagent and the primary amines found in proteins (N-terminus and ε amines in lysines). This reaction can be affected by protein samples chemically modified through their primary amines. Substances including NHS- and sNHS-biotin, dye conjugates, and TMT labeling reagents are incompatible with the assay, while amine-containing buffers, such as Tris, are typically compatible (see Table 1) for sample preparation.



Contents and storage

Materials	Quantity		Contents	Storage
	Cat. No. A50668 (100 assays)	Cat. No. A50669 (500 assays)		
Standard 1 (0 mg/mL BSA)	5 mL	5 mL	0.9% NaCl with 0.05% sodium azide	15–30°C
Standard 2 (10 mg/mL BSA)	5 mL	5 mL	10 mg/mL Bovine Serum Albumin (BSA) in 0.9% NaCl with 0.05% sodium azide	
Qubit™ Protein BR Assay Buffer	20 mL	100 mL	Buffered solution	
Qubit™ Protein BR Assay Reagent	3 mL	5 × 3 mL	Labeling reagent in acetonitrile	

Materials required but not provided

- Thin-wall, clear, 0.5-mL PCR tubes or Qubit™ assay tubes (500 tubes, Cat. No. Q32856)
- Qubit™ 4 Fluorometer

Important protocol information

Incubation Time	To allow the Qubit™ Protein BR Assay to reach optimal fluorescence, incubate the tubes for a minimum of 10 minutes after mixing the sample or the standard with the Qubit™ Protein BR Assay Reagent.
Assay Reagent	After the incubation period, the fluorescent signal is stable for 1 hour at room temperature.
Sample Volume	The Qubit™ Protein BR Assay is designed to use 10 µL or 20 µL samples. For best results, use the largest volume possible.
Qubit™ 4 Fluorometer Calibration	For each Qubit™ assay, you can run a new calibration or use the values obtained with the previous calibration. When using the instrument for the first time, perform a new calibration each time. As you become familiar with the assays, the instrument, and your pipetting accuracy, you can then determine if a new calibration is required with each use. It is recommended that a Sample Blank be run whenever a new sample type or sample buffer is being measured.
Note: This assay is only compatible with the Qubit™ 4 Fluorometer.	

Protocol

Prepare standards and samples

This protocol includes preparation of standards for calibrating the Qubit™ 4 Fluorometer.

1. Label the required number of 0.5-mL tubes for standards, buffer blanks, and samples. The Qubit™ Protein BR Assay requires two standards and one Sample Buffer blank. The option is available to read multiple replicates of Standard 2 to improve assay reproducibility. If desired, prepare additional tubes of Standard 2. The calibration will use the average relative fluorescence units (RFU) for the Standard 2 reads.

   Note: Do not label the side of the tube as this could interfere with the sample read. Label the lid of each standard tube correctly. Calibration of the Qubit™ 4 Fluorometer requires the standards and Sample Buffer blank to be inserted into the instrument in the order specified by the instrument software.
2. Prepare standards by adding 20 µL of each Qubit standard (Standard 1 and Standard 2) and 150 µL of Qubit™ Protein BR Assay Buffer to the appropriate tubes. Pipette up and down to mix.
3. Prepare samples by adding 10 µL or 20 µL of each protein sample to your sample tubes. Bring the total volume to 170 µL by adding 150 µL or 160 µL of Qubit™ Protein BR Assay Buffer to the appropriate tubes. Pipette up and down to mix.
4. Prepare the Sample Buffer Blank by adding 10 µL or 20 µL of buffer (same used to store your samples) into the appropriate tube. The volume used (10 µL or 20 µL) should match the volume used to prepare your samples. Bring the total volume to 170 µL by adding 150 µL or 160 µL of Qubit™ Protein BR Assay Buffer. Pipette up and down to mix.
5. Add 30 µL of Qubit™ Protein BR Assay Reagent to the sample tubes, standards, and Sample Buffer Blank, then mix immediately using pipetting and vortexing for 5–7 seconds. The final volume in each tube should be 200 µL.

   Note: **Upon addition of the Qubit™ Protein BR Assay Reagent, the solution may turn slightly cloudy. After pipetting and vortex mixing, the solution will become clear with a slight yellow color. The yellow color is not indicative of the amount of protein in the sample. The fluorescent protein reagent will quickly hydrolyze to a non-fluorescent product when no amines are present .**
6. Allow all tubes to incubate at room temperature for 10 minutes.

Read standards and samples

1. On the home screen of the Qubit™ 4 Fluorometer, press **Protein** then press **Protein Broad Range**. The **Read standards** screen is displayed. Press **Read standard** to proceed.

   Note: If you have already performed a calibration for the selected assay, the instrument prompts you to choose between reading new standards and running samples using the previous calibration. If you want to use the previous calibration, skip to step 5 below.
2. Insert the tube containing Standard 1 into the sample chamber, close the lid, then press **Read standard**. When the reading is complete (~3 seconds), remove Standard 1.
3. Insert the tube containing Standard 2 into the sample chamber, close the lid, then press **Read standard**. When the reading is complete, remove Standard 2. The option is available to read multiple tube replicates of Standard 2.
4. After all Standard 2 reads are complete, press **Run samples**.
5. On the assay screen, select sample volume and units:
   1. Press the **+** or **–** buttons on the wheel to select sample volume added to the assay tube (10 µL or 20 µL).
   2. From the drop-down menu, select the units (**µg/mL**, **mg/mL**) for the output sample concentration.
6. Insert the tube containing the Sample Buffer Blank into the sample chamber, close the lid, then press **Read tube**.
7. Insert sample tube into the sample chamber, close the lid, then press **Read tube**. When the reading is complete (~3 seconds), remove the sample tube. The instrument displays the results on the assay screen. The value displayed is the concentration of the sample.
8. Repeat step 7 until all samples have been read.

Interfering substances

- Certain substances are known to interfere with the Qubit™ Protein BR Assay. Substances containing primary amines, including glycine, ethanolamine, and other free amino acids may interfere. Refer to Table 1 for a list of interfering substances.
- Substances that increase the viscosity of the sample, such as high concentrations of glycerol and sucrose, may interfere with the reagent mixing. The ability to mix in a timely manner is important for measurement consistency.
- Using the sample blank addresses many of the known incompatibilities by adjusting the standard curve based on the background signal of the sample buffer. Sample buffer response which is >5-fold higher than observed fluorescent response of Standard #1 is indicative of a substance that is reacting with the Qubit™ Protein BR Assay Reagent. Background interference from Tris-containing buffers (e.g., Tris-buffered saline (TBS)), along with many other substances, is effectively eliminated by using the appropriate sample blank.

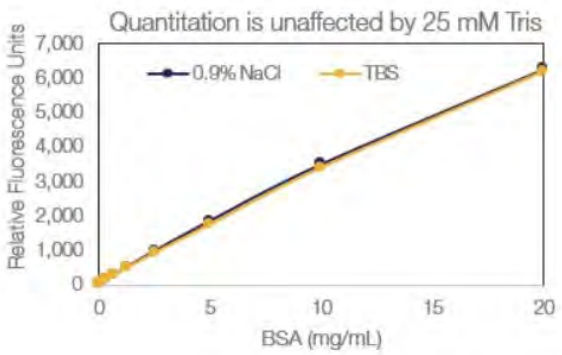


Fig. 1 Protein samples in TBS are accurately measured in the Qubit™ Protein BR Assay. BSA standards (20 mg/mL–150 µg/mL) prepared in 0.9% NaCl and Tris-buffered saline (25 mM Tris, 150 mM NaCl, pH 7.2) were measured in the Qubit™ Protein BR Assay using the protocol as described above.

- Certain denaturants (i.e., guanidine and urea) may change the number of solvent-accessible lysine residues available to the labeling reagent and result in a different observed concentration when compared to the observed concentration of the protein in its native configuration.
- Sample preparation strategies can be used to eliminate or reduce the effects of incompatible substances by using:
  - Dialysis or gel filtration.
  - Sample dilution until the substance no longer interferes.

Assay compatibility for substances and samples in the Qubit™ Protein BR Assay

Table 1 A 1 mg/mL BSA solution was prepared in the below solutions and compared to a 1 mg/mL BSA solution in 0.9% NaCl and 0.05% NaN_3. Substances were compatible at the indicated concentration if the error in protein concentration estimation caused by the presence of the substance was less than or equal to 10%. Compatibility was determined using the listed solutions as a blank during the calibration steps.

Test Compound	Compatible Concentration	Test Compound	Compatible Concentration
2-mercaptoethanol	1 mM	NE-PER (NER)	Neat
Acetonitrile	20%	NP-40	5%
Ammonium sulfate	200 mM	PBS	Neat
Bicine	100 mM	PMSF	1 mM
Borate (50mM, pH 8.5)	Neat	RIPA	Neat
B-PER™	Neat	SDS	5%
CHAPS	5%	Sodium acetate	100 mM
Carbonate-bicarbonate	Neat	Sodium chloride	5 M
Cell Surface Protein Isolation Kit	Neat	Sucrose	20%
DTT	5 mM	T-PER™	Neat
DMSO	10%	Tricine	50 mM
EDTA	50 mM	TBS	Neat
Glucose	1 M	Tris-glycine	Not compatible[1]
Glycerol	10%	Tris-glyciine SDS	Not compatible[1]
GPCR Extraction and Stabilization Reagent	4 M	Tris-HCl	500 mM
Guanidine-HCl	4 M	Tris-HEPES SDS	Neat
Imidazole	200 mM	Triton™ X-100	5%
I-PER™	Neat	Tween-20™	3%
Mem-PER™	Neat	Urea	3 M
MES	125 mM	Y-PER, undiluted	Not compatible[1]
MOPS	100 mM		
M-PER™	Neat		
NE-PER™ (CER)	Neat		

[1] Not compatible with assay at the lowest level tested.

Additional assay considerations

- It is possible to have a substance-additive effect. We recommend validating the compatibility of your specific sample buffer components.
- For the most accurate concentration determination, prepare multiple dilutions of the sample, such as 4X (10 µL + 30 µL deionized water) and 8X (10 µL + 70 µL deionized water), and then take the average concentration from all of the obtained measurements. Consider the dilution factor used when calculating the final concentration of the sample.
- This assay is not suitable for antibody concentration. We recommend the Pierce™ BCA Protein Assay (Cat. No. 23225) or absorbance measurement at 280 nm.

Related products

Product	Cat. No.
Qubit™ assay tubes, 500 tubes	Q32856
Pierce™ BCA Protein Assay Kit	23225
Qubit™ Protein Assay Kit, 100 assays, for use with Qubit™ 4 and Flex Fluorometers	Q33211
Qubit™ Protein Assay Kit, 500 assays, for use with Qubit™ 4 and Flex Fluorometers	Q33212

Troubleshooting

Observation	Possible cause	Recommended action
Protein concentration is lower or higher than expected.	Interfering substance was in sample buffer.	Desalt sample.
		Dilute sample in water to reduce the amount of interfering substance.
Replicate samples have high variability.	Tube was not mixed immediately after addition of Qubit™ Protein BR Assay Reagent.	Add Qubit™ Protein BR Assay Reagent quickly, mix 2–3 times with pipetting, cap tube, and vortex for 5 seconds.
Large discrepancies in protein concentrations are seen between different sample dilutions.	Interfering substances were in either the protein sample or sample buffer.	Desalt, dialyze, or dilute the sample even more (e.g., 10X or 20X) to remove the interfering components.

Limited product warranty

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For descriptions of symbols on product labels or product documents, go to thermofisher.com/symbols-definition.

Revision history: Pub. No. MAN0024790

Revision	Date	Description
B.0	14 June 2021	Corrected the reagent name in step 5 of “Prepare standards and samples” on page 2.
A.0	12 February 2021	New manual.