Quant-iT™ Protein Assay Kit (Q33210)

Quick Facts

Storage upon receipt:

- ≤6ºC
- Protect from light
- Avoid freeze/thaw cycles

Ex/Em: 470/570 nm

Number of assays: 1000, with a 200 µL assay volume

Introduction

The Quant-iT™ Protein Assay Kit makes protein quantitation easy and accurate. The kit provides concentrated assay reagent, dilution buffer, and pre-diluted BSA standards. Simply dilute the reagent 1:200, load 200 µL into the wells of a microplate, add 1–20 µL sample volumes, mix, then read the fluorescence (Figure 1). The assay is highly selective for protein. In the range of 0.25–5 µg of protein, the response curve is sigmoidal (pseudo-linear from 0.5–4 µg) and exhibits low protein-to-protein variation (Figure 2). The assay is performed at room temperature, and the signal is stable for 3 hours. Common contaminants, such as salts, solvents, or DNA, but not detergents, are well tolerated in the assay.

Materials

Contents

- **Component A:** Quant-iT protein reagent, 1.0 mL of a 200X concentrate in 1,2-propanediol
- **Component B:** Quant-iT protein buffer, 250 mL
- **Component C:** BSA standards, set of eight, 500 µL each of 0, 25, 50, 100, 200, 300, 400, and 500 ng/µL

Sufficient materials are supplied for 1000 assays, based on a 200 µL assay volume in a 96-well microplate format. The Quant-iT protein assay can be adapted for use in cuvettes or 384-well microplates. The Quant-iT protein reagent is a new formulation of Molecular Probes' NanoOrange® reagent.

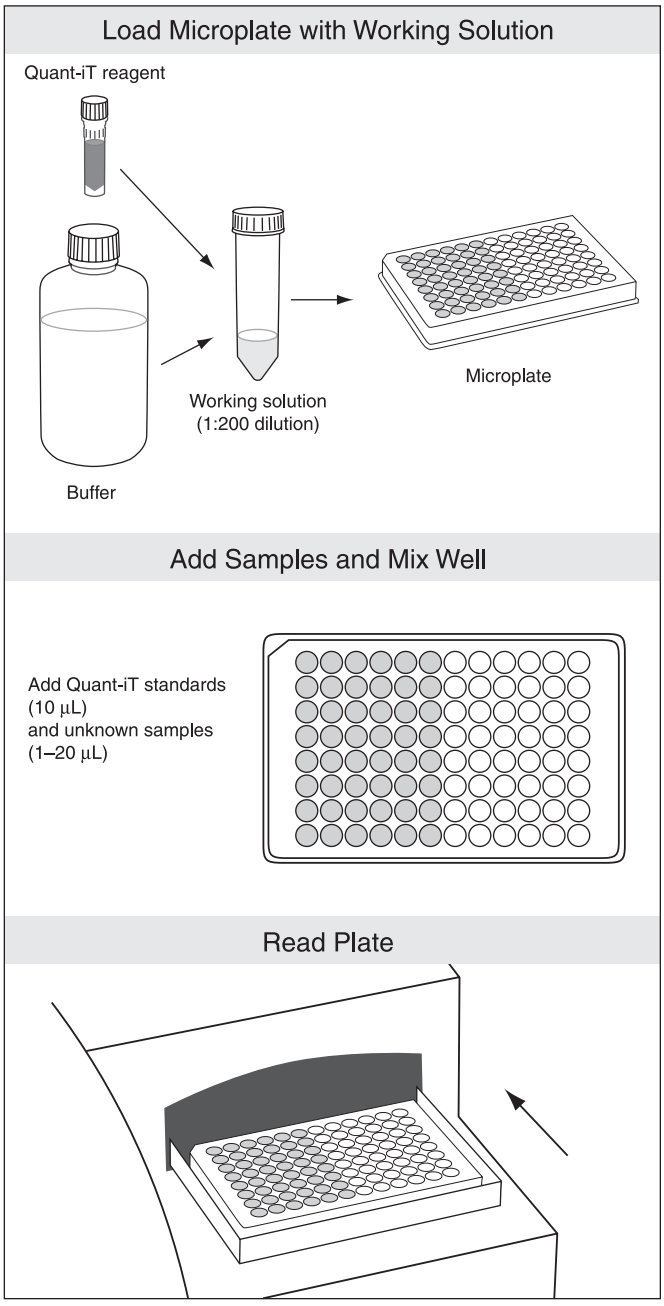

Figure 1. The Quant-iT protein assay.

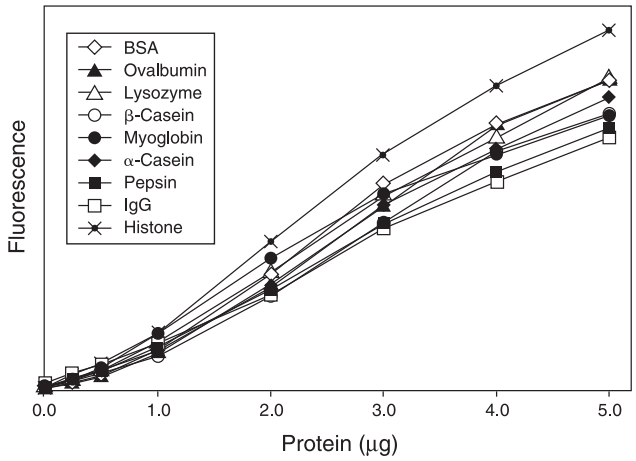

Figure 2. Lack of protein-to-protein variation in the Quant-iT protein assay. Solutions of the following proteins were prepared, diluted, and assayed in the Quant-iT protein assay: bovine serum albumin (BSA), chicken-egg ovalbumin, chicken-egg lysozyme, bovine-milk β-casein, equine myoglobin, bovine-milk α-casein, porcine pepsin, mouse immunoglobin (IgG), and calf-thymus histone. Fluorescence was measured at 485/590 nm and plotted versus the mass of the protein sample. At 3 µg, the fluorescence variation was 12.4%, or 8.7% excluding the basic histone protein. Background fluorescence has not been subtracted.

Storage

Upon receipt store the kit at ≤6ºC, protected from light. Under these conditions the components should be stable for at least 6 months. For convenience, the Quant-iT protein reagent (Component A) may be stored indefinitely at room temperature, protected from light. For short-term storage (days), the buffer (Component B) may also be left at room temperature; however, for longer periods we recommend storage at ≤6°C to prevent microbial contamination.

Protocol

During all steps, protect the Quant-iT protein reagent concentrate and the working solution from light as much as possible.

1. Equilibrate the assay components to room temperature. Remove the Quant-iT Protein Assay Kit from storage and allow the components to equilibrate to room temperature.

2. Make a working solution by diluting Quant-iT protein reagent 1:200 in Quant-iT protein buffer. For example, for ~100 assays put 100 µL of Quant-iT protein reagent (Component A) and 20 mL of Quant-iT protein buffer (Component B) in a disposable plastic container and mix well. Do not use glass containers.

3. Load 200 µL of the working solution into each microplate well. Diluted Quant-iT protein reagent is stable for at least 3 hours at room temperature, protected from light.

4. Add 10 µL of each BSA standard (Component C) to separate wells and mix well. Duplicates or triplicates of the standards are recommended.

5. Add 1–20 µL of each unknown protein sample to separate wells and mix well. Duplicates or triplicates of the unknown samples are recommended. Some contaminating substances may interfere with the assay, see below.

6. Measure the fluorescence using a microplate reader (excitation/emission maxima are 470/570 nm). The fluorescence signal is stable for 3 hours at room temperature.

7. Use a standard curve to determine the protein amounts. For the BSA standards, plot amount vs. fluorescence, and fit a curve to the data points.

Protocol Details

Reagent dilution

Buffers other than the provided Quant-iT protein buffer buffer should not be used for Quant-iT protein reagent dilution. The Quant-iT protein reagent concentrate is designed to be diluted 1:200 with the Quant-iT protein buffer (e.g. 100 µL plus 20 mL) — technically, a 201-fold dilution. Dilutions of 200-fold (e.g. 100 µL plus 19.9 mL) will also work well.

Sample volumes

Sample volumes may range from 1–20 µL. The discrepancy in final volume, 201 vs. 220 µL, has negligible consequence with most instruments. If desired, and for highest precision, the volumes of all reactions can be equalized by adding a small volume of the dilution buffer. Equalizing the volumes is important in cases where contaminating substances may be present (see below).

Assay time and temperature

The assay temperature is "room temperature," defined here as 20–25°C. Assay temperatures outside of this range have not been tested, but may be acceptable. For best results pre-incubate the reactions for 30 minutes at room temperature; after that, the fluorescence signal is stable (±10%) for at least 3 hours. In the period from 0–30 minutes the signal may be slightly higher, but accurate when compared to standards incubated for the same time.

Standard curves and extended ranges

In this manual we have plotted standard curves as mass (µg) of protein vs. fluorescence. Alternatively, the x-axis can be expressed in concentration units, as the final concentration in the assay or as the concentration of the added sample. Table 1 is provided to facilitate these unit conversions.

The fluorescence of the Quant-iT protein reagent bound to protein results in a sigmoidal standard curve from 0–5 µg (Figure 3). For best results fit a curve through the data points of the standards, including the background data point. The curve is pseudo-linear from 0.5–4 µg, and a straight line may be fit to this range

Table 1. Mass-to-Concentration Conversion. *

Protein Mass (µg)	Final Concentration µg/mL	Concentration (µg/µL) in Sample Volumes of		
		1 µL	10 µL	20 µL
0.25	1.25	0.25	0.025	0.012
1	5	1	0.1	0.05
5	25	5	0.5	0.25

* The Quant-iT protein assay is designed to detect 0.25–5 µg of protein in a 200 µL assay volume. Sample volumes may vary from 1–20 µL; therefore, sample concentration may vary from 0.01–5 µg/µL (0.01–5 mg/mL).

of data points. When 10 µL volumes of the standards are used, the lowest BSA-containing standard represents 0.25 ng of protein.

To assess the reliability of the assay in the low range, use smaller volumes of the standards, e.g. 2 µL volumes for a standard curve ranging from 0–1 µg (Figure 3, inset). During development of the Quant-iT protein assay, Molecular Probes scientists were able to detect 0.1 µg of BSA under ideal experimental circumstances (using calibrated pipettors, sextuplicate determinations, the best microplate readers, and Z-factor[1] analysis). Your results may vary.

Protein-to-protein variation

The Quant-iT protein assay has been applied to a number of different protein species, and there is minimal protein-to-protein variation in the response (Figure 3). Only the highly basic protein, histone, behaves aberrantly. Considering all proteins, assayed as 3 µg-samples, the CV in fluorescence signal is only 12.4%, or 8.7% if histone is excluded.

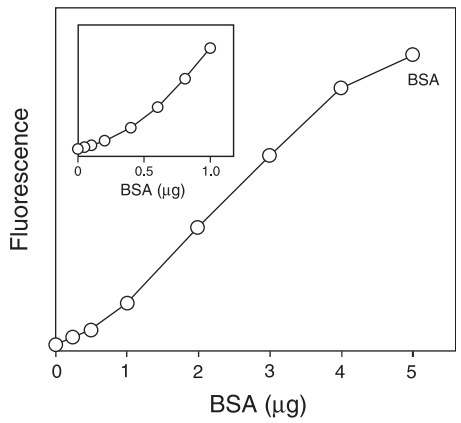

Figure 3. Protein sensitivity of the Quant-iT protein assay. The provided BSA standards were assayed in the Quant-iT protein assay using quadruplicate 10 µL or sextuplicate 2 µL volumes (inset). Fluorescence was measured at 485/590 nm and plotted versus the mass of protein in the sample. The variation (CV) of replicate protein determinations was ≤5% for the 10 µL and ≤10% for the 2 µL samples. Background fluorescence has not been subtracted.

Contaminating substances

A number of common contaminants have been tested in the Quant-iT protein assay, and most are well tolerated; however, the samples containing detergents are not recommended (Table 2). For untested contaminating substances, and, in general, for highest accuracy, the standards should be assayed under the same conditions as the unknowns. For example, if the experimental samples are in an unusual buffer and if 10 µL volumes of these samples are used, then add 10 µL volumes of the unusual buffer (lacking protein) to the assays of the standards.

Excitation and fluorescence emission maxima

The excitation and emission maxima for the Quant-iT protein reagent bound to protein are 470 and 570 nm, respectively (Figure 4). In order to maximize the signal from the fluorophore, it is usually best to choose filters that are spectrally separated and slightly offset from the peak excitation and emission wavelengths.

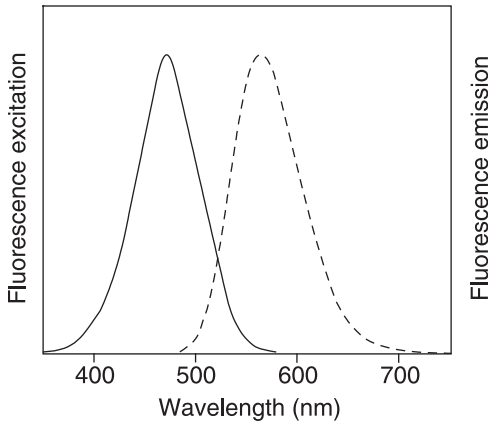

Figure 4. Excitation and emission maxima for the Quant-iT protein reagent bound to BSA.

Table 2. Effect of Contaminants in the Quant-iT Protein Assay.*

Contaminant	Final Concentration in the Assay	Concentration in 20 µL Sample	Concentration in 10 µL Sample	Result
Sodium chloride	20 mM	200 mM	400 mM	OK†
Magnesium chloride	2 mM	20 mM	40 mM	OK
Potassium chloride‡	20 mM	200 mM	400 mM	OK
Calcium chloride‡	2 mM	20 mM	40 mM	OK†
Ammonium sulfate	5 mM	50 mM	100 mM	OK†
DTT	1 mM	10 mM	20 mM	OK
β-Mercaptoethanol	1 mM	10 mM	20 mM	OK
EDTA	1 mM	10 mM	20 mM	OK
Sodium azide	1 mM	10 mM	20 mM	OK
HEPES, pH 7.4	5 mM	50 mM	100 mM	OK
Potassium phosphate, pH 7.4	5 mM	50 mM	100 mM	OK
NaCl/K–PO4, pH 7.4	1/15 mM	10/150 mM	20/300 mM	OK†
Sucrose	50 mM	500 mM	1 M	OK
Sucrose	100 mM	1 M	2 M	NR
Glycerol	1%	10%	20%	OK†
SDS	0.01%	0.1%	0.2%	OK†
SDS	0.02%	0.2%	0.4%	NR
Tween® 20	0.001%	0.01%	0.02%	NR
Triton® X-100	0.001%	0.01%	0.02%	NR
Amino acids§	100 µg/mL	1 mg/mL	2 mg/mL	OK
dNTPs**	100 µM	1 mM	2 mM	OK†
DNA	5 µg/mL	50 µg/mL	100 µg/mL	OK†
DNA	10%††	10%††	10%††	OK
DNA	50%††	50%††	50%††	NR

***** BSA standards were assayed in the presence or absence of contaminants at the indicated final concentrations. Equivalent concentrations (approximate) in 20 µL or 10 µL sample volumes are also listed. Results are given either as OK, usually less than 10% perturbation, or as NR, not recommended. **†** An acceptable result, but with some distortion of the standard curve. For best results, add the same amount of contaminant to the standard samples. **‡** A precipitate was observed. **§** A mixture of 19 amino acids. ****** A mixture of dATP, dCTP, dGTP, and dTTP. **††** For each data point, the DNA mass was a fixed percentage of the protein mass.

Reference

1. J Biomol Screen 4, 67-73 (1999).

Product List *Current prices may be obtained from our Web site or from our Customer Service Department.*

Cat #	Product Name	Unit Size
Q33210	Quant-iT™ Protein Assay Kit *1000 assays*	1 kit

Contact Information

Further information on Molecular Probes' products, including product bibliographies, is available from your local distributor or directly from Molecular Probes. Customers in Europe, Africa and the Middle East should contact our office in Leiden, the Netherlands. All others should contact our Technical Assistance Department in Eugene, Oregon.

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Molecular Probes, Inc.
29851 Willow Creek Road, Eugene, OR 97402
Phone: (541) 465-8300 • Fax: (541) 335-0504

Customer Service: 6:00 am to 4:30 pm (Pacific Time)
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Phone: (541) 335-0353 • Toll-Free (800) 438-2209
Fax: (541) 335-0238 • tech@probes.com

Molecular Probes Europe BV
Poortgebouw, Rijnsburgerweg 10
2333 AA Leiden, The Netherlands
Phone: +31-71-5233378 • Fax: +31-71-5233419

Customer Service: 9:00 to 16:30 (Central European Time)
Phone: +31-71-5236850 • Fax: +31-71-5233419
eurorder@probes.nl

Technical Assistance: 9:00 to 16:30 (Central European Time)
Phone: +31-71-5233431 • Fax: +31-71-5241883
eurotech@probes.nl