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iQuant™ Protein Assay Kit

(12.5 µg/mL – 5 mg/mL)

Catalog Number: N030, N031

Table 1. Kit Components and Storage

Material	Amount	Concentration	Storage	Stability
iQuant™ Protein Assay Kit (Cat. No. N030)				The product is stable for one year when stored as directed.
iQuant™ Protein Reagent (Component A)	300 µL	200X in 1,2-propanediol	2-8 °C Protect from light	
iQuant™ Protein Buffer (Component B)	50 mL	1X		
Protein Standard #1 (Component C)	1 mL	0 ng/µL		
Protein Standard #2 (Component D)	1 mL	200 ng/µL		
Protein Standard #3 (Component E)	1 mL	400 ng/µL		
iQuant™ Protein Assay Kit (Cat. No. N031)				
iQuant™ Protein Reagent (Component A)	1.5 mL	200X in 1,2-propanediol	2-8 °C Protect from light	
iQuant™ Protein Buffer (Component B)	250 mL	1X		
Protein Standard #1 (Component C)	5 mL	0 ng/µL		
Protein Standard #2 (Component D)	5 mL	200 ng/µL		
Protein Standard #3 (Component E)	5 mL	400 ng/µL		

Approximate fluorescence excitation/emission maxima, in nm: 470/570.

Product Description

The iQuant™ Protein Assay Kit provides a simple, quick, and accurate quantitation for protein samples. The kit contains concentrated assay reagent, dilution buffer, and pre-diluted BSA standards. Simply dilute the concentrated assay reagent using the buffer provided, add your sample (any volume from 1-20 µL is acceptable), then read the concentration using the Qubit™ Fluorometer. The assay is accurate for initial sample concentrations from 12.5 µg/mL to 5 mg/mL and exhibits low protein-to-protein variation. The assay is performed at room temperature, and the signal is stable for 3 hours. Common contaminants such as reducing reagents (DTT, β-mercaptoethanol), salts, free nucleotides, amino acids, solvents, or DNA (but not detergents) are well tolerated in the assay.

Handling and Disposal

There is no safety data available for iQuant™ Protein Reagent. Treat the iQuant™ Protein Reagent with the same safety precautions as other potentially harmful reagents and to dispose of the reagent in accordance with local regulations. Centrifuge the iQuant™ Protein Reagent and the BSA standards before opening vials to minimize loss on the cap. Use properly calibrated pipettes for best accuracy.

General Protocol

1. Measure protein samples using a Fluorescence Microplate Reader

1.1 Warm up the iQuant™ Protein Assay Kit to room temperature.

1.2 Prepare the iQuant™ working solution by diluting the iQuant™ protein reagent 1:200 in iQuant™ protein buffer. Use a clean plastic tube each time you make iQuant™ working solution. For example, to measure 8 samples in duplicate, add 20 μL of iQuant™ protein reagent to 4 mL of iQuant™ protein buffer. Mix well and use immediately.

1.3 Prepare a series of protein standard dilutes from Protein Standard #3 (Component E).

1.4 Add 190 μL of the iQuant™ working solution to each well of a black 96-well microplate used for standards. Black plates such as Greiner or Corning black 96-well plates are recommended to minimize fluorescence bleed-through from other well.

1.5 Add 10 μL of each protein standard dilutes in duplicate or triplicates into separated wells and mix well.

1.6 Add the iQuant™ working solution to individual wells used for samples so that the final volume in each well after adding sample is 200 μL.

Note: Your sample can be anywhere from 1–20 μL. Add a corresponding volume of iQuant™ working solution to each well: anywhere from 180-199 μL.

1.7 Add each sample to the wells containing the correct volume of the iQuant™ working solution and mix well. The final volume in each well should be 200 μL.

1.8 Incubate the microplate at room temperature for 15 minutes in the dark.

1.9 Measure the fluorescence using a microplate reader with 470 nm excitation and 570 nm emission, with the appropriate cut-off.

1.10 Use a standard curve to determine the protein concentrations. For protein standards, plot amount vs fluorescence, and fit a straight line to the data points.

2. Measure protein samples using the Qubit® Fluorometer

2.1. Warm up the iQuant™ Protein Assay Kit to room temperature.

2.2. Prepare the iQuant™ working solution by diluting the iQuant™ protein reagent 1:200 in iQuant™ protein buffer. Use a clean plastic tube each time you make iQuant™ working solution. For example, to measure 8 samples in duplicate, add 20 μL of iQuant™ protein reagent to 4 mL of iQuant™ protein buffer. Mix well and use immediately.

2.3. Add 190 μL of the iQuant™ working solution to each assay tube used for standards. (**Note:** Use only thin-wall, clear 0.5 mL PCR tubes. Axygen PCR-05-C tubes (VWR, Cat No. 10011-830)).

2.4. Add 10 μL of protein standard #1, protein standard #2, and protein standard #3 to the appropriate tubes, then mix by vortexing 2-3 seconds, and label the lids of each protein standard tube correctly. Be careful not to create bubbles.

2.5. Add the iQuant™ working solution to individual tubes used for samples so that the final volume in each well after adding sample is 200 μL.

Note: Your sample can be anywhere from 1–20 μL. Add a corresponding volume of iQuant™ working solution to each well: anywhere from 180-199 μL.

2.6. Add each sample to the tubes containing the correct volume of the iQuant™ working solution, and mix by vortexing 2-3 seconds. The final volume in each tube should be 200 μL.

2.7. Incubate all tubes at room temperature for 15 minutes in the dark.

2.8. Choose the **Protein** program on the Qubit® fluorometer and calibrate the Qubit® fluorometer using standard #1, standard #2, and standard #3.

2.9. Read the user samples in the Qubit® fluorometer.

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Appendix

Table 2. Effect of Contaminants in the iQuant™ Protein Assay Kit

Contaminant	Final Concentration in Assay	Concentration in 10 µL Sample	Result
Sodium Chloride	20 mM	400 mM	OK
Magnesium Chloride	2 mM	40 mM	OK
Ammonium Sulfate	5 mM	100 mM	OK
EDTA	1 mM	20 mM	OK
Sodium Azide	1 mM	20 mM	OK
DTT	1 mM	20 mM	OK
β-Mercaptoethanol	1 mM	20 mM	OK
Potassium Phosphate, pH 7.4	5 mM	100 mM	OK
Sucrose	50 mM	1 M	OK
Glycerol	1%	20%	OK
Imidazole	1.25 mM	25 mM	OK
Sodium Dodecyl Sulfate	0.01%	0.2%	OK
Sodium Dodecyl Sulfate	0.02%	0.4%	NR
Triton X-100	0.001%	0.02%	NR
Tween 20	0.001%	0.02%	NR
dNTPs	100 µM	2 mM	OK
Amino Acids	0.1 mg/mL	2 mg/mL	OK
DNA	5 µg/mL	100 µg/mL	OK

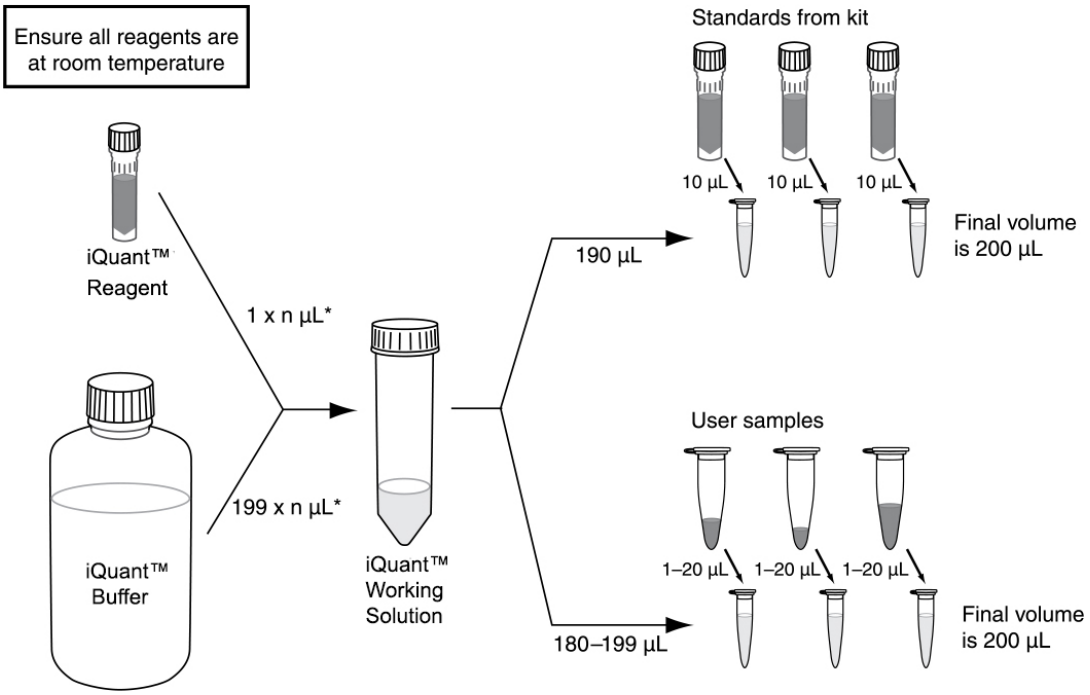

Figure 1. iQuant™ Protein Assay Workflow