Thermo SCIENTIFIC

PROTOCOL NanoDrop 3300

Quant-it™ Protein Assay

Introduction

The Quant-iT™ Protein dye is a fluorescent stain for quantitating minute amounts of protein in solution. When activated with the blue LED (470nm), the fluorescence of the protein-dye complex has an emission wavelength range of 590-610nm. Unlike general UV A280 absorbance spectroscopy, the Quant-iT™ protein dye is able to circumvent the contributions from free amino acids, interfering absorbing molecules such as nucleic acid, and common contaminating salts yielding only signal from total protein (see product information sheet for further details). Using as little as 2ul per measurement, the NanoDrop 3300 allows significantly reduced reaction volumes requiring a fraction of the total volume needed for conventional cuvette-based fluorometers.

Dynamic Range

The Quant-iT™ Protein assay is designed to measure total mass per reaction. This protocol relates the total mass to concentration as the sample data is reported in units of ug/ml. The largest dynamic range for the assay can be obtained by utilizing two distinct working reagent concentrations. The 25ng/ul - 500ng/ul range is covered using a twenty fold (20X) diluted working reagent. An eighty fold (80X) diluted working reagent is preferred for the 5ug/ml - 100ug/ml per reaction lower range. When using the Quant-iT™ Protein working reagents at the above concentrations, sigmoidal curves are produced and are best fit using the 3rd order polynomial option on the NanoDrop 3300 Fluorospectrometer.

Quant-iT™ Protein Assay Supplies

Equipment:

- NanoDrop 3300 Fluorospectrometer
- 2uL pipettor (low retention nuclease free tips)

Materials:

- Low lint laboratory wipes
- Amber or foil covered 1.5ml polypropylene tubes

Reagents:

- Quant-iT™ Protein assay kit (Molecular Probes Part# Q33210) Includes 8 BSA standards (0-500 µg/ml)

Quant-iT™ Protein Assay Recommendations

- Mix all solutions gently to avoid micro bubbles.
- Remove samples from the optical surfaces by blotting rather then wiping as this will reduce residual lint fibers from collecting on the NanoDrop 3300 Fluorospectrophotmeter sampling pedestals.

Quant-iT™ Protein Assay

1. Equilibrate the Quant-iT™ Protein buffer, Quant-iT™ Protein reagent concentrate and protein standards to room temperature. *Protect the reagent from light.*

2. Mix both the concentrated Quant-iT™ Protein reagent and the buffer thoroughly.

3. Proceed to either the High range or Low range protocol.

High Range Protocol (25 µg/ul - 500 µg/ul Protein)

a. To prepare the Quant-iT™ Protein working solution dilute the stock Quant-iT™ Protein reagent twenty (20X) fold by transferring 190ul of Quant-iT™ protein buffer and 10 ul of the concentrated reagent to an amber tube and mixing thoroughly but gently. *Prepare fresh as the diluted Quant-iT™ Protein working solution is stable for only a few hours. Do note use glass containers.*

b. Transfer 20ul of the Quant-iT™ Protein working solution into amber tubes (one per each standard and unknown sample).

c. Mix each stock BSA standard solution thoroughly and transfer 10ul of each BSA standard to the respective amber tube to obtain a total reaction volume of 30ul .

Stock BSA conc. (µg/ml)	BSA volume (ul)	Quant-iT™ working solution (diluted 20X)	BSA (assay) (µg/ml)
500	10	0	500
400	10	0	400
300	10	0	300
200	10	0	200
100	10	0	100
50	10	0	50
25	10	0	25
0	10	0	0

Range Protocol (cont)

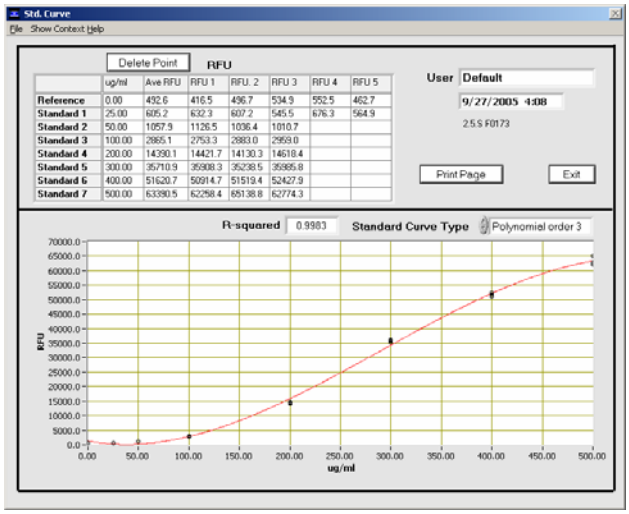
d. Pipette 10ul of the unknown samples to the appropriate amber tube containing 20 ul of the Quant-iT™ protein working solution. If using less then 10ul of an unknown sample, add the corresponding volume of Quant-iT™ protein buffer to make a final ***sample*** volume of 10ul.

e. Mix each standard and unknown sample thoroughly, collect the solution at the bottom of the tube by a brief centrifugation, and allow the reaction to incubate at room temperature for 30 minutes. The final reaction volume for each standard and unknown should be 30ul. *Note: Run the assay as soon as possible after the incubation period as the signal is only stable for only a few hours.*

f. Mix well, collect the solution at the bottom of the tube by a brief centrifugation, and proceed to the ND-3300 standard curve protocol on page 3.

High Range Standard Curve

Figure 1. Example of the operating software display of a high range standard curve.



	ug/ml	Ave RFU	RFU 1	RFU 2	RFU 3	RFU 4	RFU 5
Reference	0.00	492.6	416.5	496.7	534.9	552.5	462.7
Standard 1	25.00	605.2	632.3	607.2	545.5	676.3	564.9
Standard 2	50.00	1057.9	1126.5	1036.4	1010.7		
Standard 3	100.00	2885.1	2753.3	2883.0	2959.0		
Standard 4	200.00	14390.1	14421.7	14130.3	14618.4		
Standard 5	300.00	35710.9	35908.3	35238.5	35985.8		
Standard 6	400.00	51620.7	50914.7	51519.4	52427.9		
Standard 7	500.00	63380.5	62258.4	65138.8	62774.3		

The standard labels in the example graph correspond to the actual ***concentration*** of the manufacturers standard solutions. Since 10ul of the standard is used per reaction , the total protein mass (250ng – 5000ng) is 10x each concentration.

Low Range Protocol (5ng/ul-100ng/ul Protein)

Dilute the stock Quant-iT™ protein reagent eighty (80X) fold by transferring 395ul of Quant-iT™ protein buffer and 5 ul of the concentrated reagent to an amber tube and mixing thoroughly but gently. (Note: Prepare fresh as the diluted Quant-iT™ protein working solution is stable for only a few hours. Do note use glass containers.)

b. Transfer 20ul of the Quant-iT™ protein working solution into the amber tubes (one per each standard and unknown sample).

c. Mix each BSA standard solution thoroughly and transfer 2ul of each BSA standard and 8ul of the Quant-iT™ buffer to the respective amber tube to obtain a total reaction volume of 30ul.

Table 2: Low range protein standards.

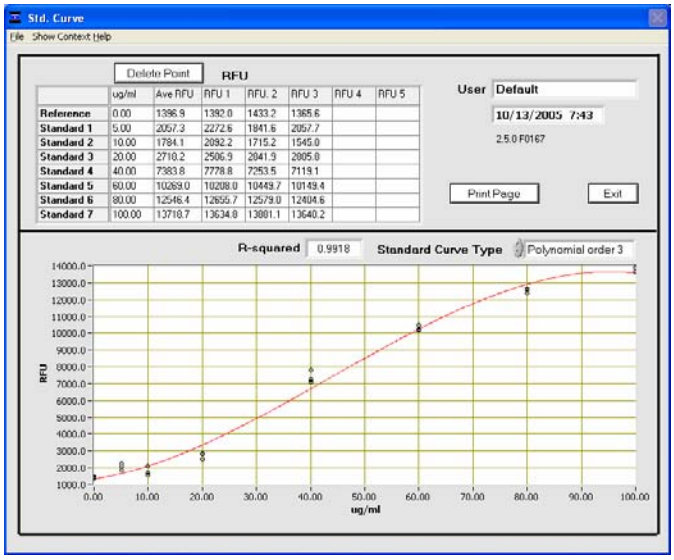
Stock BSA conc. (µg/ml)	BSA volume (ul)	Quant-iT™ buffer	BSA (assay) (µg/ml)
500	2	8	100
400	2	8	80
300	2	8	60
200	2	8	40
100	2	8	20
50	2	8	10
25	2	8	5
0	2	8	0

d. Pipette 10 ul of the unknown samples to the appropriate amber tube containing 20 ul of the Quant-iT™ protein working solution. If using less than 10ul of an unknown sample, add the corresponding volume of Quant-iT™ protein buffer to make a final sample volume of 10ul.

e. Mix each standard and unknown sample thoroughly, collect the solution at the bottom of the tube by a brief centrifugation, and allow the reaction to incubate at room temperature for 30 minutes. The final total reaction volume should be 30ul. *Note: Run the assay as soon as possible after the incubation period as the signal is only stable for only a few hours.*

f. Mix well, collect the solution at the bottom of the tube by a brief centrifugation, and proceed to the NanoDrop 3300 standard curve protocol on page 3.

Low Range Standard Curve

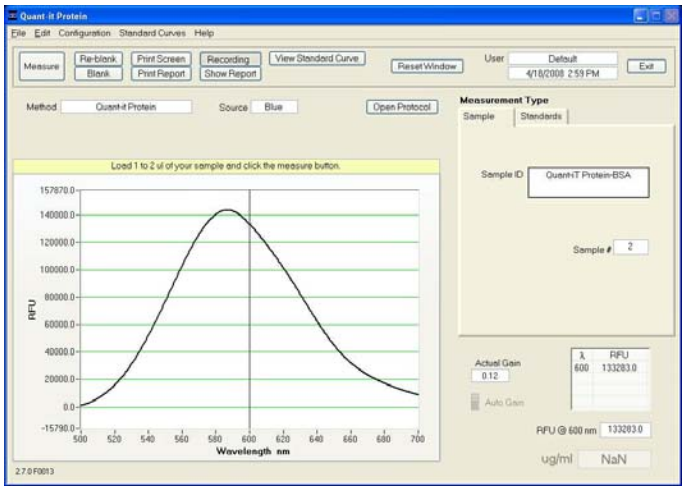


	ug/ml	Ave RFU	RFU 1	RFU 2	RFU 3	RFU 4	RFU 5
Reference	0.00	1396.9	1392.0	1433.2	1365.6		
Standard 1	5.00	2057.3	2272.6	1841.6	2057.7		
Standard 2	10.00	1784.1	2092.2	1715.2	1545.0		
Standard 3	20.00	2718.2	2506.9	2041.9	2805.8		
Standard 4	40.00	7383.8	7778.8	7253.5	7119.1		
Standard 5	60.00	10269.0	10208.0	10449.7	10149.4		
Standard 6	80.00	12546.4	12655.7	12579.0	12404.6		
Standard 7	100.00	13718.7	13634.8	13801.1	13640.2		

Figure 2. Example of the operating software display of a low range standard curve.

The standard labels in the example graph correspond to the actual ***concentration*** of the standard solutions.

Example spectrum of a Quant-it Protein sample



Standard Curve Protocol

1. Clean both sampling pedestals with 2 uL of nuclease free deionized water.

2. Open upper arm and firmly blot the two pedestals with a dry lab wipe. Make sure there are no traces of lint on the pedestals before continuing.

3. Open the operating software. Click on the Protein Quantitation button and select the Quant-it Protein method.

4. Add 2 uL of assay buffer (no dye, no sample) to the lower pedestal. Lower the arm and click F3 or the Blank button. When the measurement is complete, lift the arm and use a dry laboratory wipe to blot the buffer from both the bottom and upper measurement surfaces. Use a fresh aliquot of buffer to verify a proper baseline.

5. Under Measurement type, click on the Standards tab. Highlight the Reference standard.

6. Mix the reference solution (assay buffer and dye, no sample) briefly and transfer 2 uL of the solution onto the lower pedestal. Lower the arm and click F1 or the Measure button. A pop up window will ask for confirmation of the units. (Recommended ng/mL or pg/uL)

7. Measure up to 5 replicates of the reference solution using a fresh 2 uL aliquot for each measurement.

8. Select Standard 1 to enter a value. Enter values for up to 7 standards.

9. Mix the standard solution briefly and transfer 2 uL onto the lower pedestal. Lower the arm and click F1 or the Measure button. Measure up to 5 replicates of each standard using a fresh 2 uL aliquot for each measurement.

10. Once the standard curve is completed, select the Standard Curve Type (Interpolation, Linear, 2° polynomial, 3° polynomial) that best fits the standards data set.

11. Click on the Sample tab under Measurement Type, and enter the unknown samples' respective ID information. If a dilution of the unknown sample was made, enter the dilution factor in the box below the sample ID window.

12. Add 2 ul of the sample and use the F1 key or click the Measure button to initiate the measurement cycle. Use a fresh aliquot of sample for each measurement.