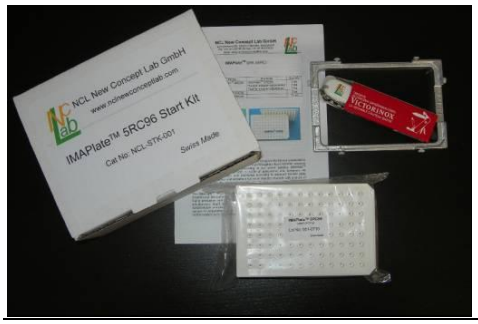

IMAPlate™ 5RC96 Application Note

Protein quantification: Bradford Protein Assay

Introduction

The Bradford protein assay is a simple and rapid method to determine the total protein concentration in a sample. The assay uses Coomassie G-250 Dye as a colorimetric reagent for the quantification of protein. In the acidic environment of the reagent mixture, the absorbance peak of the dye will change from 465 nm to 595 nm when the dye binds to protein. Within the linear range, the absorbance value at 595 nm is proportion to the total amount of protein existing in the reagent mixture.

IMAPlate™ 5RC96 is the world's first miniaturized analytical platform capable of manually performing high-throughput liquid transfer, analysis, reaction and assay. It comprises 96 identical, funnel-like reaction units positioned according to standard 96-well plate format and each reaction unit contains a 5 µl round reaction chamber with a light path of 5 mm. The bottomless reaction chamber uses capillary force to confine sample solution inside it; therefore up to 96 samples can be analyzed one-by-one in a microwell plate reader.

The use of IMAPlate™ 5RC96 for the Bradford protein assay would provide scientists an easy-to-use, miniaturized analytical tool for protein quantification. It offers benefits such as:

- minimize the consumption of delicate protein samples - only requiring 0.1 µl to 4 µl sample
- no need for time-consuming sample dilutions - flexible sample volume
- large linear measurement range - up to 4000 µg/ml
- high throughput - obtaining up to 96 individual data in one measurement
- save reagent and produce less chemical waster

Experimental

Reagents and Materials

- Bradford Reagent - Bio-Rad
- Protein standards
- Pipettes (can accurately transfer 1 and 4 µl)
- IMAPlate™ 5RC96 start kit
- Microwell plate reader (e.g. ***BioTek PowerWave™ Microplate Spectrophotometer***)

Procedure A (high concentration of protein sample)

1. Pipette 4µl of 1:4 diluted Bradford reagent (fresh prepared mixture of one part of the reagent plus 3 part of distilled water) to the reaction chambers.
2. Pipette 1µl of protein standards and sample (mixing very well before use) to the assigned reaction chambers.
3. Invert the IMAPlate™ 5RC96 several times to mix the solution.
4. Place the IMAPlate™ 5RC96 in the reader with the adaptor.
5. Measure the peak absorbance at wavelength of 595 nm and base line absorbance at wavelength of 800 nm.

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If desired, the peak absorbance and base line absorbance can also be measured at other wavelengths between 575 nm to 615 nm and 750 nm to 850 nm respectively.

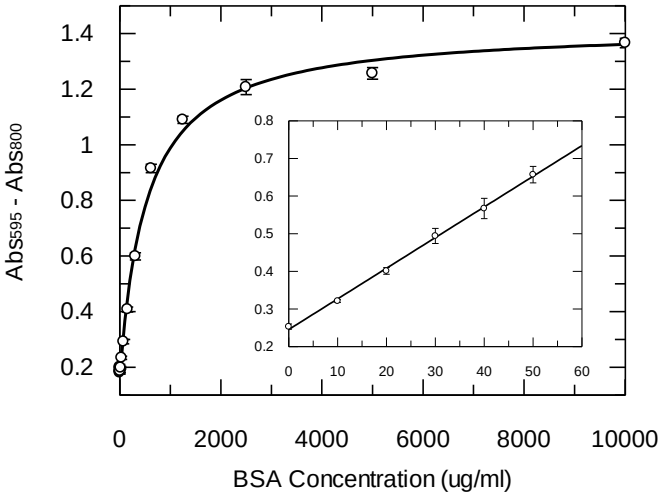
6. Use true absorbance values (A_{595} – A_{800}) to plot the standard curve and calculate the concentration of samples according to the standards.

Procedure B (low concentration of protein sample)

1. Pipette 1µl of un-diluted Bradford reagent to the reaction chambers.
2. Pipette 4µl of protein standards and sample (mixing very well before use) to the assigned reaction chambers.
3. Invert the IMAPlateTM 5RC96 several times to mix the solution.
4. Place the IMAPlateTM 5RC96 in the reader with the adaptor.
5. Measure the peak absorbance at wavelength of 595 nm and base line absorbance at wavelength of 800 nm.
6. Use true absorbance values (A_{595} – A_{800}) to plot the standard curve and calculate the concentration of samples according to the standards.

Results and Discussion

The plot of the true absorbance values against BSA concentration gives a typical binding curve as expected (see left figure). If the data are treated by graph fitting software with binding mode, the detection range of the BSA can be from 0 to 10000 µg/ml. But a straight line can be obtained in a narrow range with low concentration of protein. For example, in **Procedure A** the concentration of BSA is between 0 to 400 µg/ml or in **Procedure B** the concentration of BSA is between 0 to 100 µg/ml. the linear range also can be extended up to 4000 µg/ml if 0.1 µl of sample is mixed with 5 µl of 1:5 diluted Bradford reagent. To the current setup (the final reagent concentration is 1:5 dilution) the amount BSA in the reaction chamber from 0.04 to 0.4 µg shows a near-linear relationship with the true absorbance values.



Ordering information:

Catalog No:	Article	Contents
NCL-STK-001	IMAPlate™ 5RC96 Start Kit	5 IMAPlate™ 5RC96 plates 1 reader adaptor (adjustable) 1 tool for adaptor adjustment 1 data sheet
NCL-P5W-002	White IMAPlate™ 5RC96	5 plates / box
NCL-P5B-004	Black IMAPlate™ 5RC96	5 plates / box
NCL-P5T-006	Transparent clear IMAPlate™ 5RC96	5 plates / box
NCL-P5Y-008	Transparent yellow IMAPlate™ 5RC96	5 plates / box

Products selection:

IMAPlate™ 5RC96	Liquid transfer	Absorbance measurement	Fluorescence measurement	Reaction
White	√	UV-Vis-IR	-	√
Black	√	UV-Vis-IR	√	√
Transparent clear	√	-	-	√
Transparent yellow	√	UV	-	√