Technique Presentation: Bradford Assay

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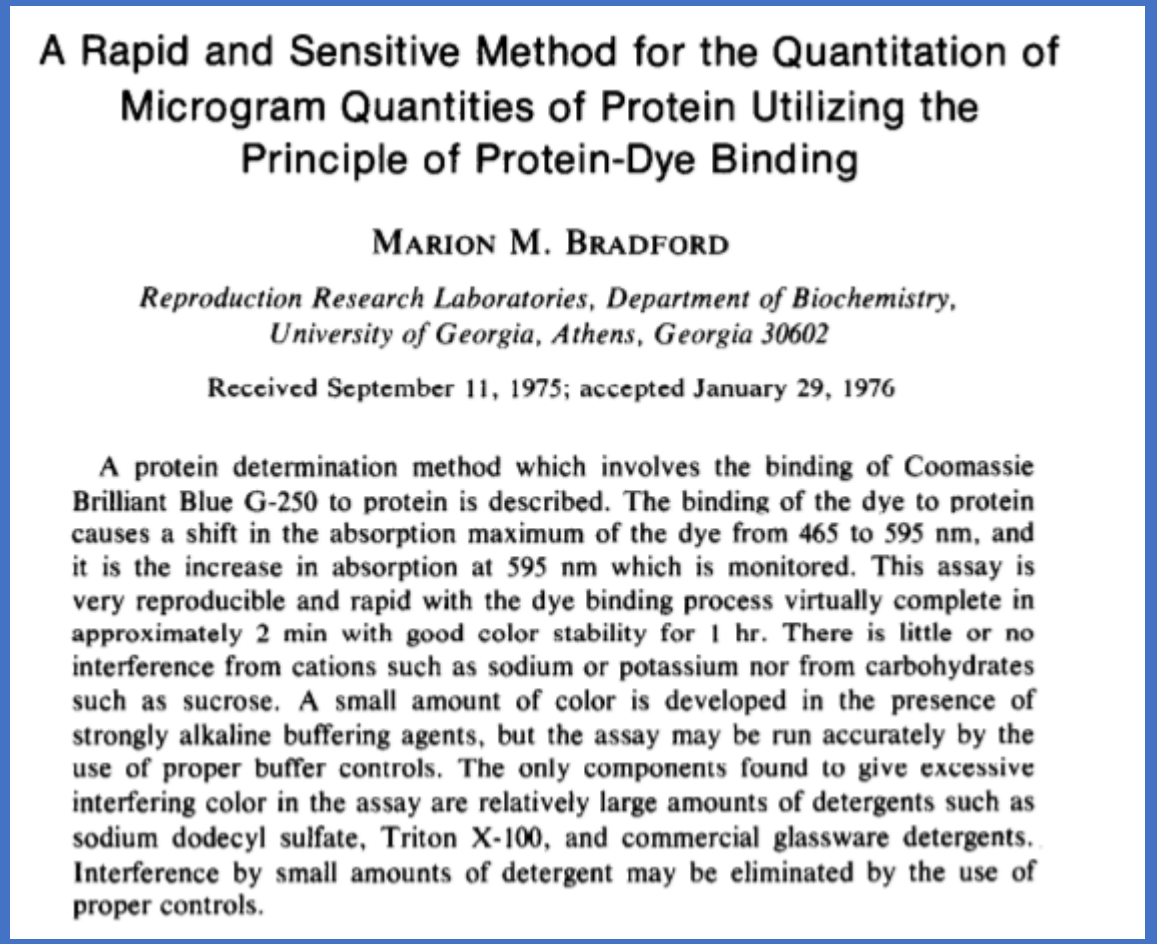
Background

- Spectroscopic analytical procedure for protein concentration determination
- Developed by Marion M. Bradford in 1976
- The Bradford reagent consists of:
  - Coomassie Brilliant Blue G-250
  - Ethanol
  - Phosphoric Acid
- Sensitivity: µg/mL



A Rapid and Sensitive Method for the Quantitation of Microgram Quantities of Protein Utilizing the Principle of Protein-Dye Binding

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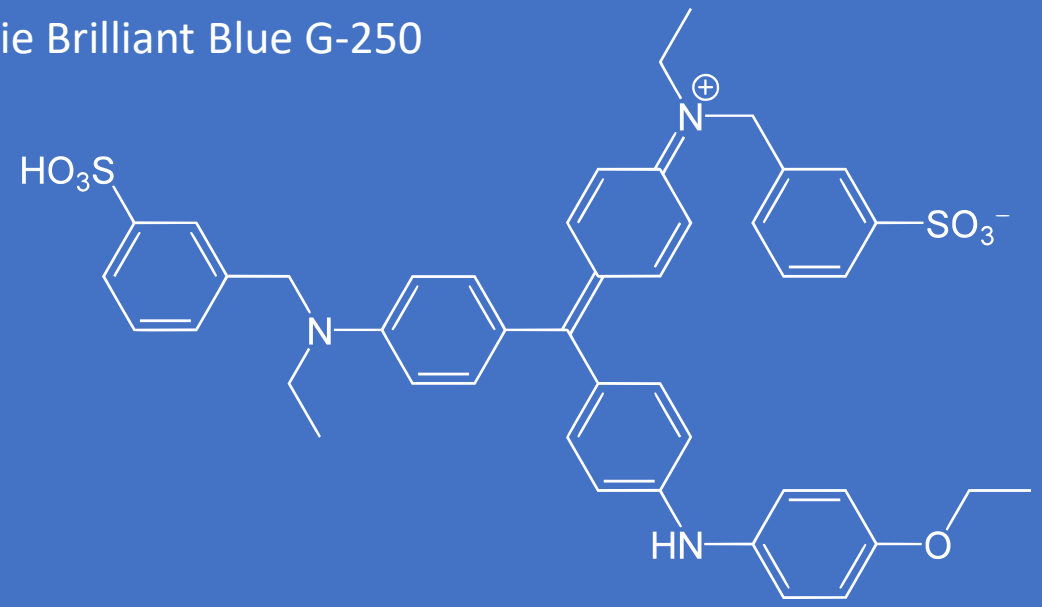
A protein determination method which involves the binding of Coomassie Brilliant Blue G-250 to protein is described. The binding of the dye to protein causes a shift in the absorption maximum of the dye from 465 to 595 nm, and it is the increase in absorption at 595 nm which is monitored. This assay is very reproducible and rapid with the dye binding process virtually complete in approximately 2 min with good color stability for 1 hr. There is little or no interference from cations such as sodium or potassium nor from carbohydrates such as sucrose. A small amount of color is developed in the presence of strongly alkaline buffering agents, but the assay may be run accurately by the use of proper buffer controls. The only components found to give excessive interfering color in the assay are relatively large amounts of detergents such as sodium dodecyl sulfate, Triton X-100, and commercial glassware detergents. Interference by small amounts of detergent may be eliminated by the use of proper controls.

Anal Biochem. **1976**,*72*, 248-254

Chemical Principle

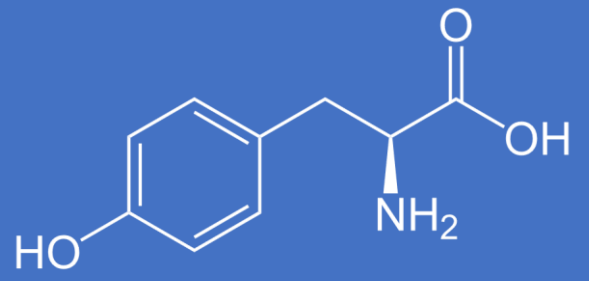
- Shift in absorbance maximum from 470 to 595 nm caused by the binding of Coomassie Brilliant Blue G-250 in acidic conditions
- Coomassie Brilliant Blue performs three types of non-covalent binding to amino acid residues:
  - Hydrophobic Interactions
  - Ionic Interactions
  - π-stacking*

Coomassie Brilliant Blue G-250



Lysine

Tyrosine



Anal Bioanal Chem. **2008**, *391*, 391–403

Limitations

- Must be performed in acidic conditions
  - Binding of Coomassie Brilliant Blue G-250 is dependent on acidic conditions, making it incompatible with certain buffers
- Not compatible with detergents(e.g. SDS)
- Relative Method
- Time Sensitive
  - The protein-dye complex aggregates over time diminishing the sample absorbance. For optimum results, measurements should be taken less than an hour after adding the Bradford Reagent
- Effectiveness Varies by Protein
  - Binding of the dye is dependent of amino acid composition, lack of certain amino acids in the analyte could lead to incorrect results.

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Methodology: Overview

Standard and Sample Preparation

Spectrophotometric Analysis

Data Analysis

Materials

- Bradford Reagent
- Pipets: 2-20µL ,20-200µL
- Eppendorf Tubes: 1.5 mL or 2.0 mL
- 96 -Well Flat Bottom Plate (Clear)
- Tecan M200 Infinite Pro Microplate Reader
- Protein Standards
- Sample

Methodology: Standard and Sample Preparation

- Standard Preparation
    1. Prepare a 20 mg/mL stock protein solution
    2. Measure the stock solution absorbance using the nanodrop to determine its concentration through the Beer-Lambert equation
        - Bovine Serum Albumin $\lambda_{max:}$:280 nm Ɛ=43,824 M^{-1} cm^{-1}
        - Human Serum Transferrin $\lambda_{max:}$:278 nm Ɛ=93,000 M^{-1} cm^{-1}
    3. Prepare standards ranging from 0 to 1.25 mg/mL of protein by diluting the stock solution

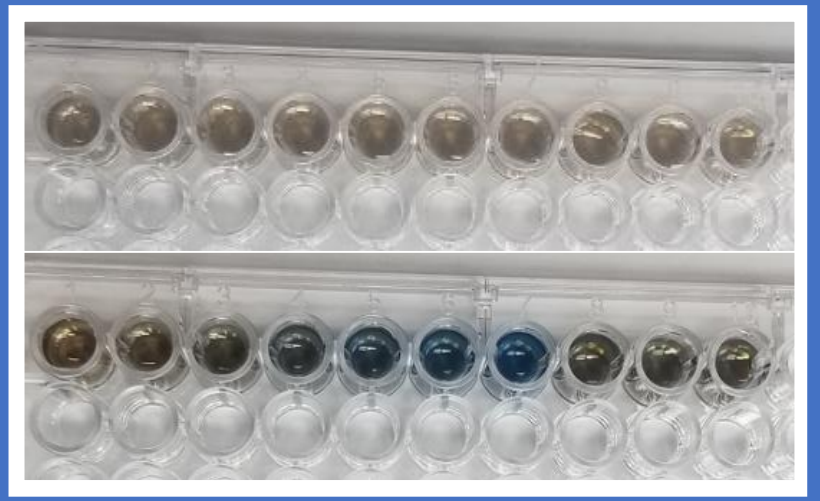
- Sample Preparation
    1. Dilute the sample to fit in the standard concentration range if necessary

Tips:

- Put buffer in first
- Avoid the formation of bubbles
- Mix thoroughly by pipetting up and down

Methodology: Reaction with the Bradford Reagent

1. Add 200µL of Bradford reagent to each well being used in the 96-Well plate
2. Add 4µL of the protein standards and the sample to separate wells
3. Mix thoroughly by pipetting up and down
4. Equilibrate standards and samples at room temperature for 5 minutes

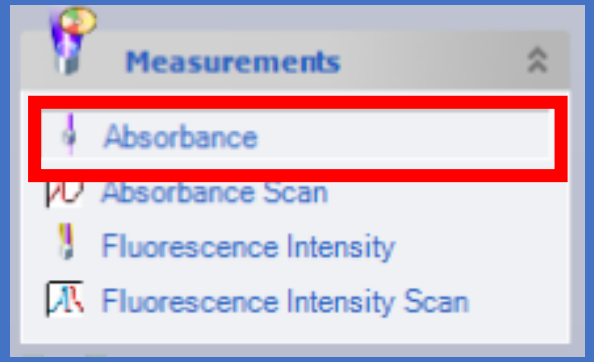


Tips:

- Bring Bradford reagent to room temperature before starting the assay
- Avoid bubble formation
- Write down the order in which you put the standards in the plate

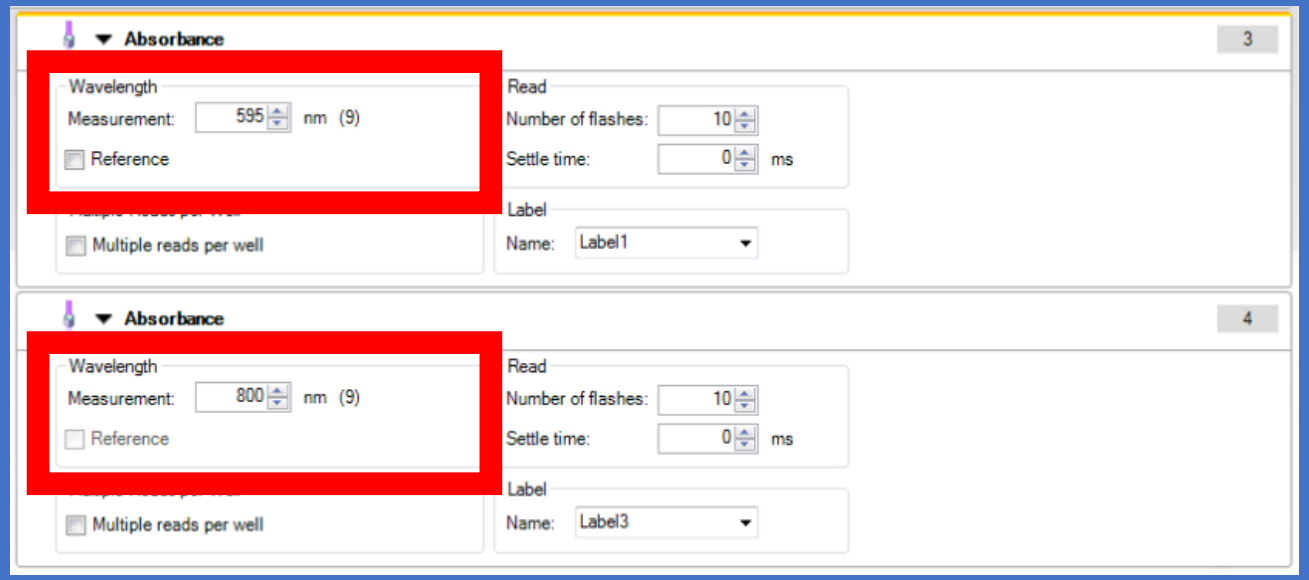
Methodology: Spectrophotometric Analysis

1. Turn on the computer, followed by the Tecan M200 Infinite Pro Microplate Reader
2. In the desktop, open the Tecan i-control program
3. Under measurements, select “Absorbance” twice
4. Under wavelength, select 595 nm and 800nm



Tips:

- Turn on the instrument before starting the assay
- Check for bubbles before measuring

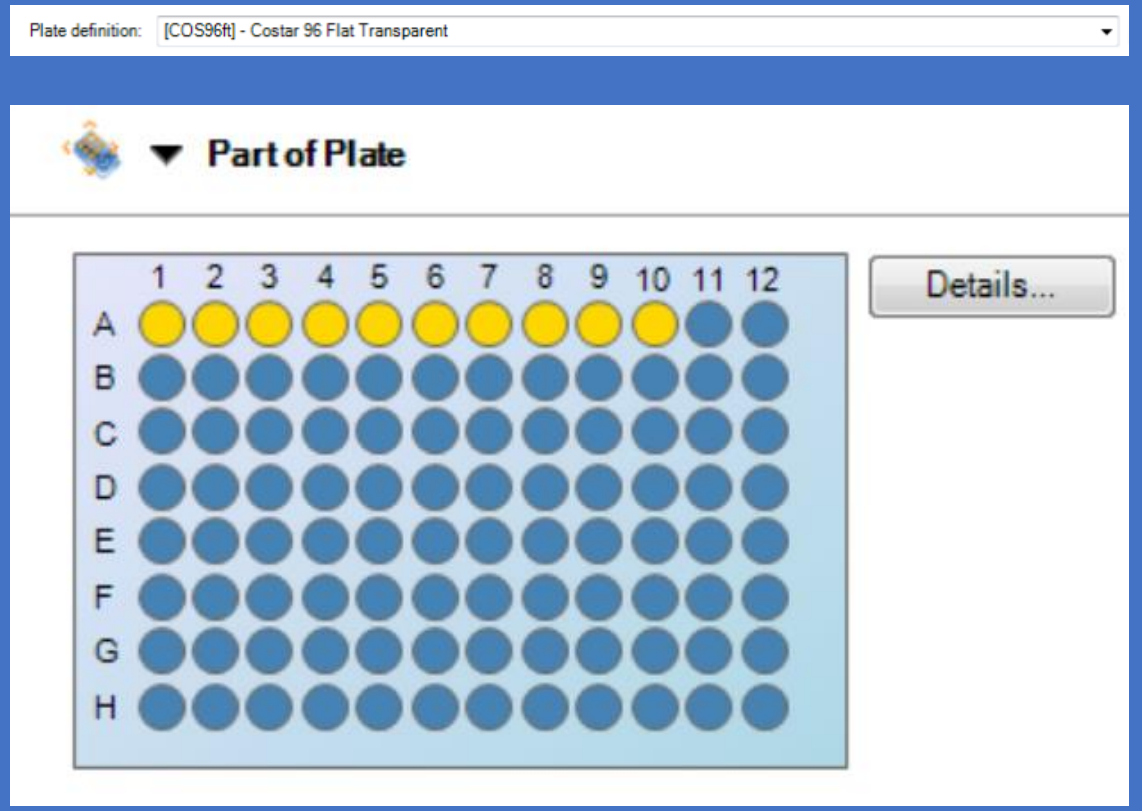


Methodology: Spectrophotometric Analysis

5. Select the plate you are using in the “Plate definition” tab
6. Select the wells in which your standards and samples are located
7. Hit “Start”
8. Collect data as an Excel™ spreadsheet

Tips:

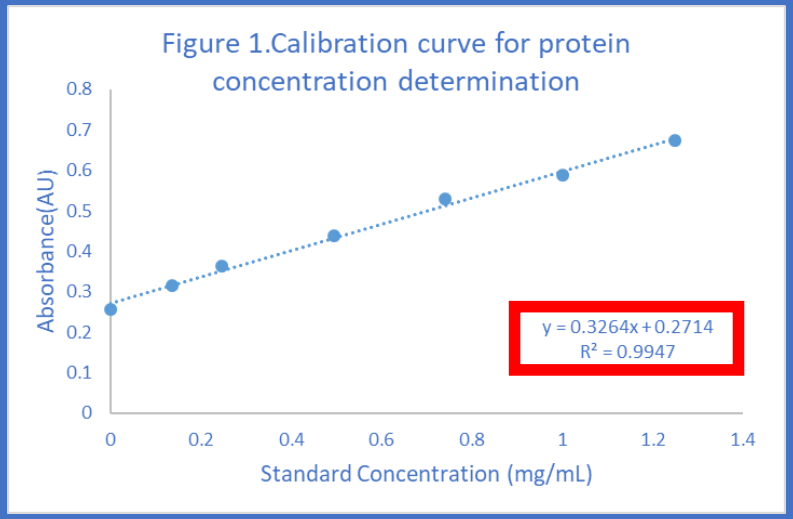
- Double check you chose the correct plate
- Corroborate your absorbance data qualitatively and do multiple reads



Methodology: Data Analysis

1. Subtract the baseline absorbance(Abs_{800}) from each of the standard and sample absorbances (Abs_{595})
2. Construct a calibration curve by plotting standard absorbance vs. standard concentration(in mg/mL)
3. Apply a lineal fit
4. Use the trend line equation to calculate the sample concentration

Table 1. Bradford assay spectrophotometric data.			
Standard Concentration (mg/mL)	Abs_{595} (AU)	Abs_{800} (AU)	Abs_{595}-Abs_{800}(AU)
0.000	0.332	0.075	0.257
0.125	0.393	0.078	0.315
0.250	0.441	0.079	0.363
0.500	0.517	0.079	0.437
0.750	0.617	0.088	0.529
1.000	0.676	0.089	0.587
1.250	0.762	0.088	0.674



Alternate Methods

LOWRY ASSAY

SPECTROSCOPY

IMMUNOASSAY

Voet,D. ;Voet,J.; Pratt, C.W. Fundamentals of Biochemistry,3rd ed.; John Wiley & Sons, Inc.