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Got Protein?

Testing the protein content of common foods

Bradford Protein Assay

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Got Protein?

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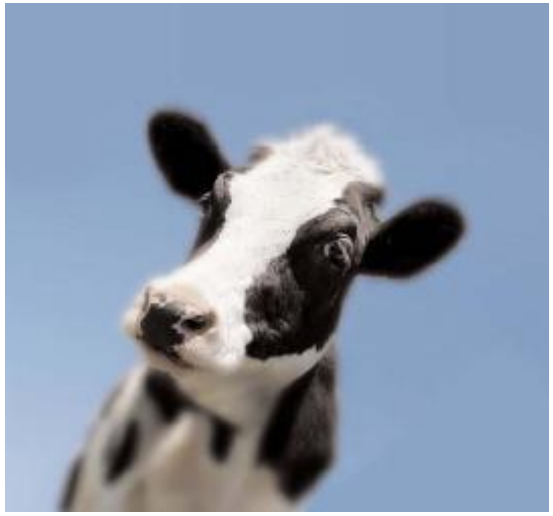
Why Teach

Got Protein?



- Powerful teaching tool
- Laboratory extensions
- Real-world connections
- Link to careers and industry
- Interdisciplinary – connects physics, chemistry and biology
- Standards based

Got Protein? Kit – Core Content Alignment	
Scientific Inquiry •Quantitation of milk proteins •Use of a spectrophotometer •Use of experimental controls •Creation and use of a standard curve	**Chemistry of Life** •Chemical and physical properties of proteins •Biophotonics and Beer's Law •Protein chemistry and structure •Chemistry of dye molecules •Properties of chemical bonds
Cell and Molecular Biology •Protein production and secretion •Nutrition and immunity	**Environmental and Health Science** •Lactose •Mineral and vitamin requirements
Evolution •Function of milk proteins •Role of milk in reproductive success of organisms •Natural Selection	**Genetics** •DNA>RNA>protein>trait •Biochemistry of milk

Got Protein? Kit Advantages

- **Explore biophotonics**
- **Study protein structure/function**
- **Learn and apply Beer's law**
- **Learn spectrophotometry**
- **Construct and use standard curves**
- **Measure protein concentrations**
- **Sufficient materials for 80 student work stations (4 students per station)**

Workshop Time Line

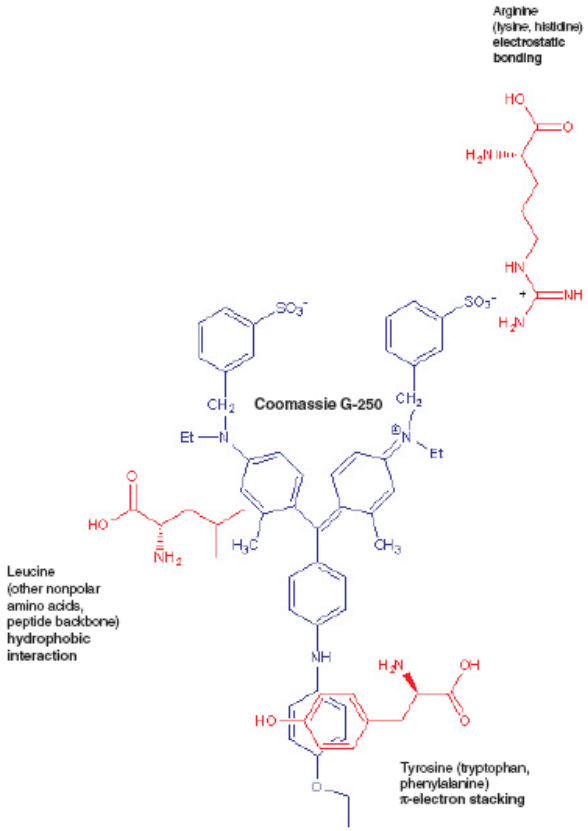
- **Introduction**
- **Review of the Bradford Test**
- **Prepare Protein Standards and Samples**
- **Measuring Absorbance and Generate a Standard Curve**
- **Determine Protein Concentrations of Unknowns**
- **Laboratory Extensions**

Why measure protein concentration?

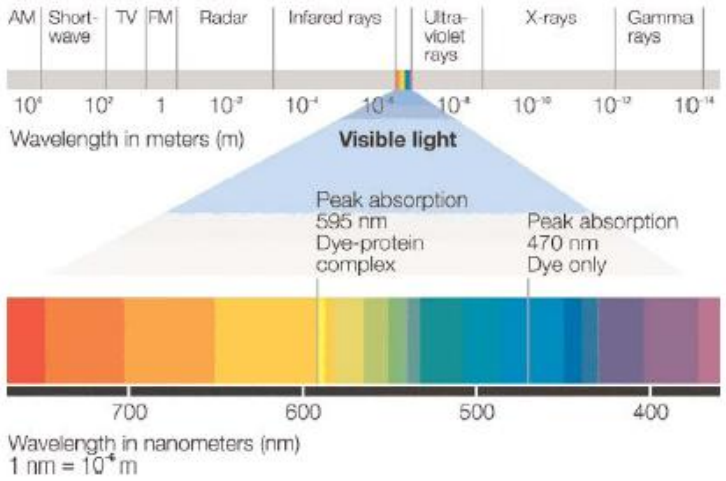
- **First step of research protocols for chromatography, electrophoresis, western blotting**

- **Sample quantitation**
  - Forensics
  - Toxicology
  - Allergens
  - Pharmacology
  - Food

Bradford Assay



- **Uses Coomassie Blue dye which binds to the side chains of specific amino acids**
- **Shifts the absorbance from 470nm (reddish-brown) to 595nm (blue)**
- **Intensity of blue correlates with concentration of protein, measure:**
  - Qualitatively by eye
  - Quantitatively with a spectrophotometer



Beer's Law

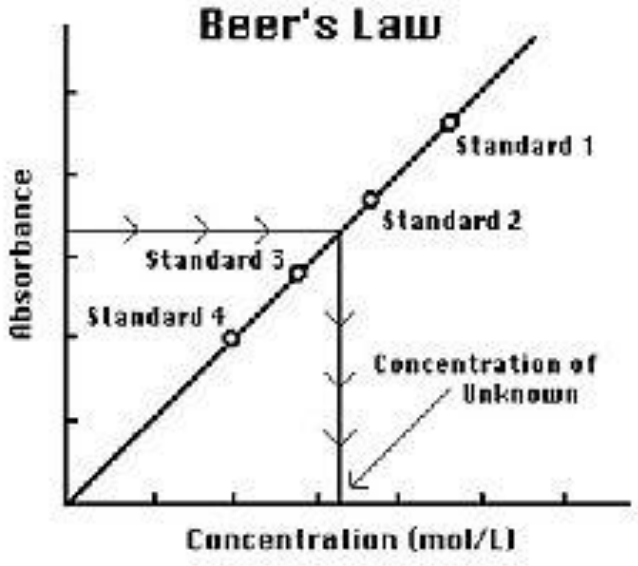
$A=\varepsilon bc$

If a solute absorbs light of a particular wavelength, the absorbance is directly proportional to the concentration of that solute in solution up to a point.

ε - the molar absorbtivity (L mol-1 cm-1)

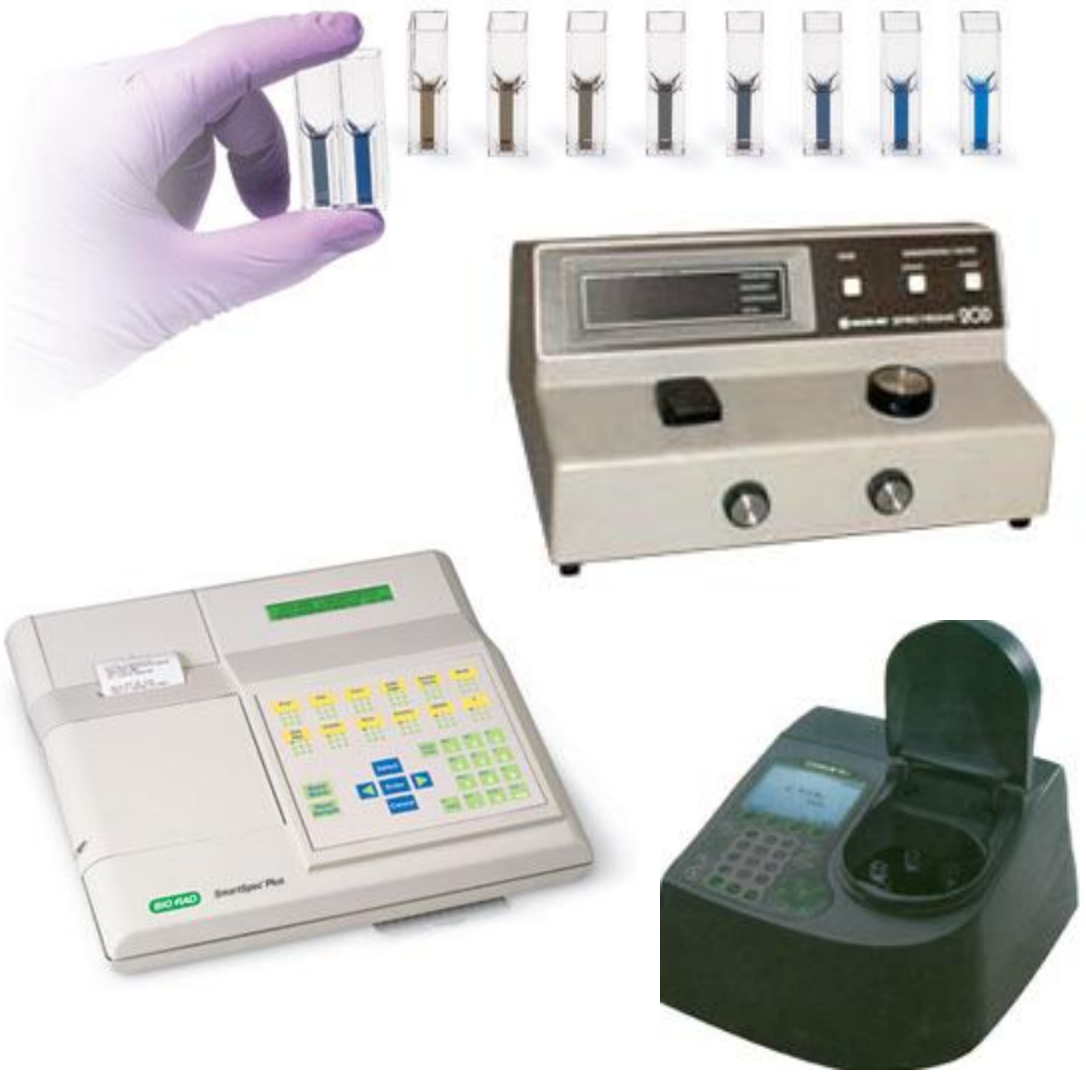
b - the path length of the sample (usually 1cm-cuvette)

C - the concentration of the compound in solution (mol L-1)



Measuring Absorbance

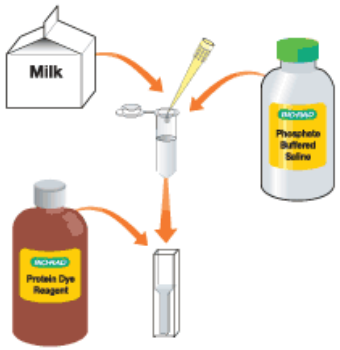
Spectrophotometers



Procedures Overview

Prepare test samples for spectral analysis

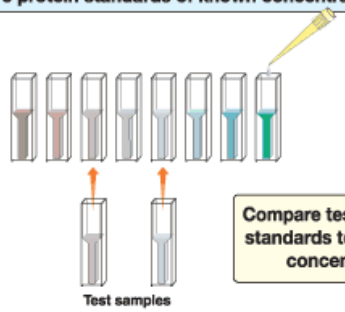
Dilute test samples of unknown protein concentration 1:50 in phosphate buffered saline



Add 20 µl diluted test samples and 1 ml protein dye reagent to cuvettes

Prepare protein standards of known concentration

Add 20 µl of a series of protein standards of known concentration to cuvettes

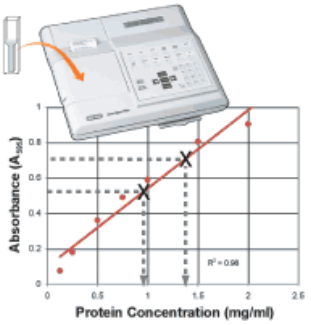


Add 1 ml protein dye reagent to each cuvette

Compare test samples to protein standards to estimate unknown concentrations by eye

Read protein standards and test samples in spectrophotometer

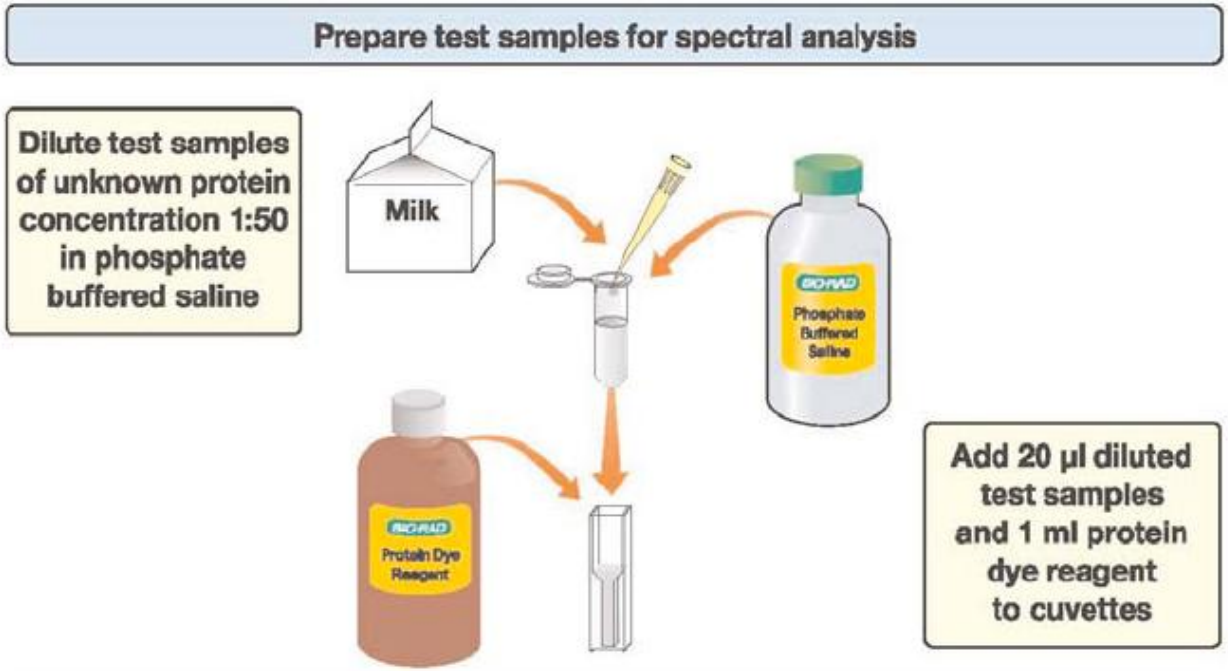
Generate standard curve from protein standards' absorbance data



Determine protein concentrations of test samples from the standard curve

Compare test samples' true protein concentration to published product labels

Make Sample Dilutions

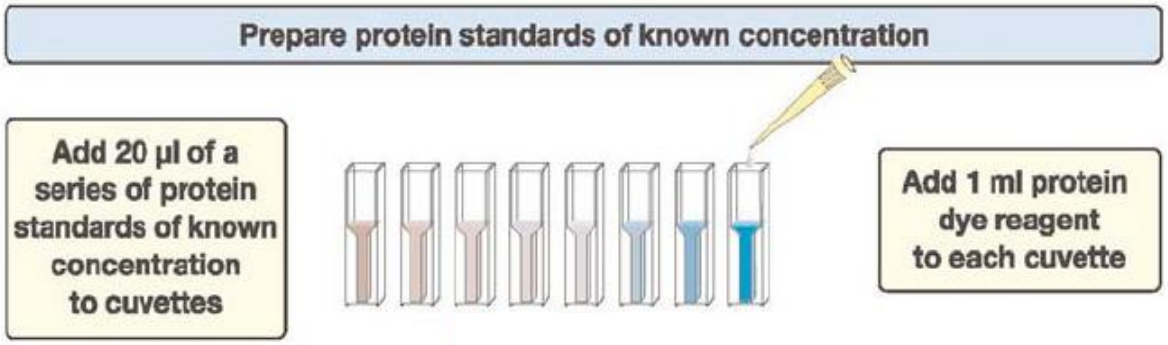


- **Prepare a 1:50 dilution of the two milk samples using 1xPBS:**
  - **Sample A**
  - **Sample B**

Add Coomassie Dye

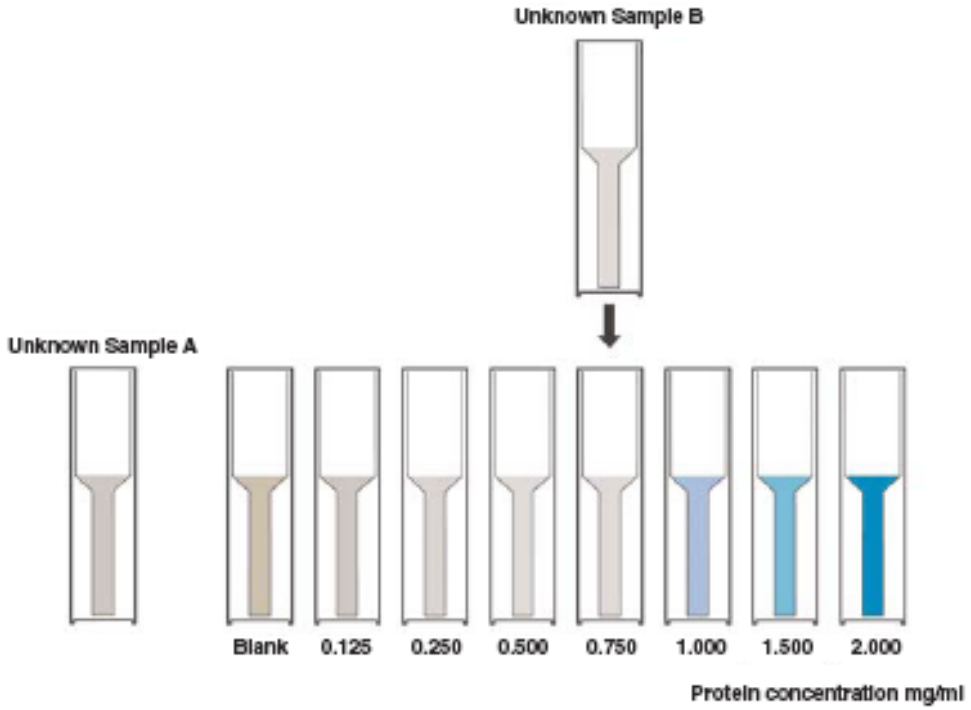
Label cuvettes (in mg/ml):

blank 1x PBS
1 0.125
2 0.250
3 0.500
4 0.750
5 1.000
6 1.500
7 2.000
A Sample A
B Sample B



- **Add 1 ml of coomassie dye to each cuvette**
- **Using a fresh tip for each sample, pipet 20 µl of each standard into the appropriate cuvette (20 µl of 1xPBS for "blank"). Then pipet 20 µl of each diluted milk sample into the appropriate cuvette.**
- **Cover each cuvette with parafilm and invert each 3x to mix.**
- **Incubate at room temperature for a period of at least 5 minutes (but not to exceed 60 minutes).**

Qualitative Determination of Protein Concentrations



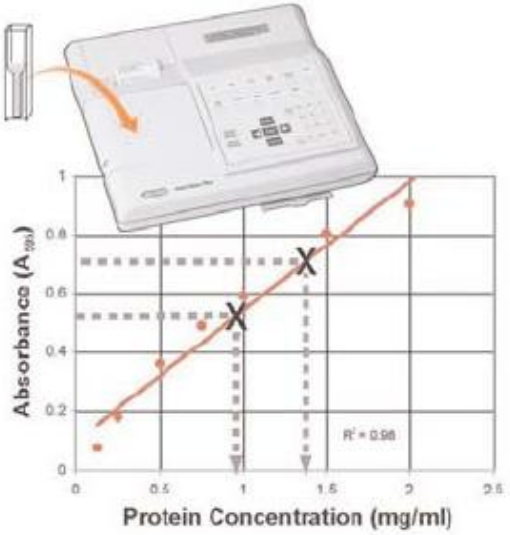
- **Visually compare the color of the unknown samples (A and B) against the standards of known concentration.**

Quantitative Determination of Protein Concentrations

Read Samples Analyze Results

Read protein standards and test samples in spectrophotometer

Generate standard curve from protein standards' absorbance data



Determine protein concentrations of test samples from the standard curve

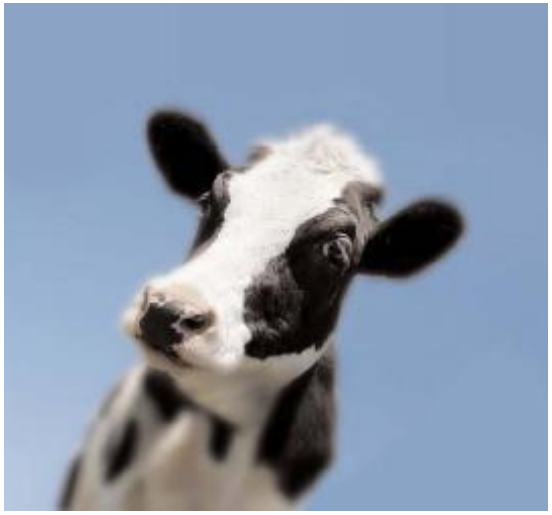
- Read the A_{595} for each standard and generate a standard curve with the data
- Determine the protein concentrations of Sample A and B from the standard curve

Bradford Assay Limitations

- The assay measures total protein concentration, different methods must be used to identify specific proteins.
- Assay is linear over a limited range
- The coomassie dye binds specifically to arginine and hydrophobic amino acids.
- The amino acid composition can alter the concentration-absorbance curve. Use of a standard (like BSA-Bovine Serum Albumin) with a similar composition must be used.

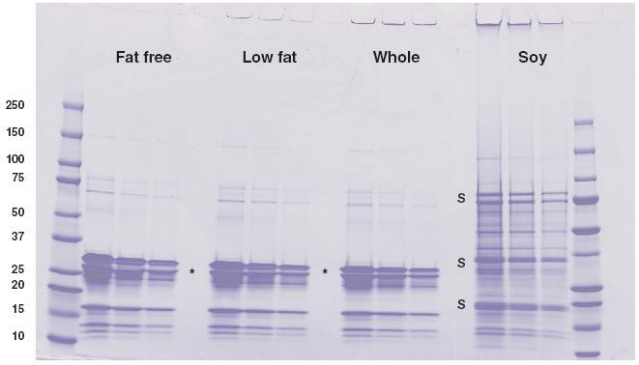
Proteins found in milk

Got Protein?



- **Major proteins unique to milk are:**
  - **Caseins**
  - **Whey proteins**
- **Caseins are important for the growth and development of the nursing young**
- **The major whey proteins in cow milk are β-lactoglobulin and α-lactalbumin which is important for lactose synthesis**
- **Other proteins found in milk are:**
  - **Immunoglobulins (antibodies)**
  - **serum albumin**
  - **enzymes**
  - **growth factors**
  - **nutrient transporters**

Laboratory Extensions



- **Determine the protein concentration of other samples:**
  - **Different types of milk**
  - **Saliva**
  - **Tears**
  - **Other food**
  - **Egg yolks vs. egg whites**
- **Analyze the specific protein content in the samples by performing SDS-PAGE and Western Blot**
- **Students prepare protein standards**

Prepare the Protein Standards

$M_1V_1 = M_2V_2$
or
$C_1V_1 = C_2V_2$

- **Construct standards or use "Quick Start" standards**

- **Constructing dilutions of known protein standards:**

To make a 0.2mg/ml sample from a 2mg/ml stock solution:

$$C_1V_1 = C_2V_2$$
$$2\text{mg/ml } (V_1) = 0.2\text{mg/ml } (1\text{ml})$$
$$V_1 = \frac{0.2\text{mg/ml } (1\text{ml})}{2\text{mg/ml}}$$
$$V_1 = 0.1\text{ml}$$

Need 0.1ml of the 2mg/ml stock solution (0.9ml of 1xPBS) to make a 0.2mg/ml sample