University of Teramo

Course of Study in Biotechnology - **Biochemical Methodologies – 2nd Year**

PRACTICAL SESSION 4 – Total Protein Quantification from Cell Lysate Using the Bradford Assay

Date: 27/11/2026

The Bradford reagent (Coomassie Brilliant Blue G-250) shifts from reddish-brown to blue upon contact with proteins; the absorbance shift from 465 nm to 595 nm is proportional to protein concentration and obeys the Beer–Lambert law within the linear working range.

Step 1: Construction of a BSA calibration curve.

Step 2: Measurement of cell lysate absorbance and calculation of protein concentration by interpolation. This value is required to normalise results from subsequent functional assays (e.g. enzymatic activity, Western blot) and to evaluate cell lysis efficiency.

Theoretical Background

✎ **Question** What is the Beer–Lambert law? Explain the physical meaning of each term. Over which protein concentration range is linearity maintained in the standard Bradford assay?

✎ **Question** Describe the interaction mechanism between Coomassie Blue G-250 and proteins. Which chemical property of amino acid residues is critical? Why is BSA chosen as the standard protein?

✎ **Question** What is a "calibration curve"? What is the coefficient of determination R^2 and what minimum value is required for your calibration curve to be considered acceptable? How is the unknown sample concentration calculated from the linear equation?

Materials and Equipment

Solutions (kept on ice):

- Bradford reagent (1×, ready to use)
- BSA stock solution (1 mg/mL in dH_2O)
- Distilled water (dH_2O)

Sample:

- Clarified cell lysate from Practical Session 2 — supernatant obtained after centrifugation

Equipment:

- UV-visible spectrophotometer (set to 595 nm)
- Ice and sample carrier tray
- Micropipettes (20, 200, 1000 µL) and tips
- 1.5 mL Eppendorf tubes
- Vortex mixer
- Polystyrene cuvettes (l = 1 cm), compatible with spectrophotometer at 595 nm

Safety and Waste Disposal

SAFETY GUIDELINES

Coomassie Blue is a dye: always wear gloves and a lab coat throughout the practical session.

The Bradford reagent may contain methanol (depending on the formulation): work in a well-ventilated area. Avoid contact with skin and eyes. If contact occurs, wash immediately with plenty of water.

Collect all Coomassie-containing waste in the designated container. DO NOT dispose of it down the sink.

Broken cuvettes: dispose of in the designated sharps container.

The mitochondrial sample is of biological origin: treat as biological hazardous waste (yellow container).

1. Preparation of the Calibration Curve

a) Preparation of standards

Starting from the 1 mg/mL BSA stock solution, prepare the dilution series shown in Table 1 in labelled Eppendorf tubes (B, S1–S6). The total volume per standard is 100 µL, made up with dH_2O.

Tube	BSA stock (µL)	dH_2O (µL)	[BSA] final (µg/mL)	Final amount (µg)
Blank (B)	0	100	0	0
S1	2	98	20	2
S2	5	95	50	5
S3	10	90	100	10
S4	20	80	200	20
S5	40	60	400	40
S6	100	0	1000	100

Table 1. BSA standards for the calibration curve

b) Addition of Bradford reagent

- Add 900 µL of Bradford reagent (ready to use) to each Eppendorf tube (B, S1–S6).
- Vortex for 3 seconds. Observe the colour shift: from reddish-brown to blue.
- Incubate at room temperature for 5 minutes.

c) Absorbance measurement at 595 nm

- Zero the spectrophotometer using the blank cuvette (B): this corrects for the background colour of the reagent.
- Transfer approximately 800 µL of each standard (S1–S6) into a clean cuvette and measure absorbance at 595 nm.
- Record A_{595} values immediately in the laboratory notebook.

2. Sample Preparation and Measurement

The sample to be quantified is the clarified cell lysate obtained at the end of Practical Session 2 (supernatant collected after centrifugation at 14,000 × g of the cell pellet lysed with lysis buffer). Since the total protein concentration is unknown, a preliminary dilution is required to fall within the linear range of the calibration curve. Keep the sample on ice throughout.

a) Sample dilution

- Prepare a 1:5 dilution: pipette 20 µL of sample + 80 µL of dH_2O into a clean Eppendorf tube.

b) Addition of reagent and measurement

- Add 900 µL of Bradford reagent to the tube containing the diluted sample.
- Vortex for 3 seconds and incubate for 5 minutes at room temperature (same timing as standards).
- Measure A_{595} in triplicate: prepare 3 separate aliquots (200 µL + 800 µL reagent each).
- Record the 3 absorbance values in the box below:

Raw data — sample
Replicate 1 — A_{595} = _____
Replicate 2 — A_{595} = _____
Replicate 3 — A_{595} = _____
Mean A_{595} = _____
SD = _____
CV% = _____

3. Data Processing (worked example in class; each student will then build their own in the lab report)

a) Construction of the calibration curve

- Plot A_{595} (y-axis) against [BSA] in µg/mL (x-axis).
- Perform linear regression: add a linear trendline and display the equation y = mx + b and the R^2 value.
- Verify that $R^2 \geq 0.99$. If not, identify outliers and discuss with the instructor.

Linear regression parameters
Slope m = _____
Intercept b = _____
R^2 = _____

Formula for the original sample:
C (original sample) = [(A_{595} − b) / m] × dilution factor
where b = intercept, m = slope of the calibration curve.

Results
Protein concentration (diluted sample) = _____
Protein concentration (original sample) = _____
Mean ± SD (triplicate) = _____
CV% = _____