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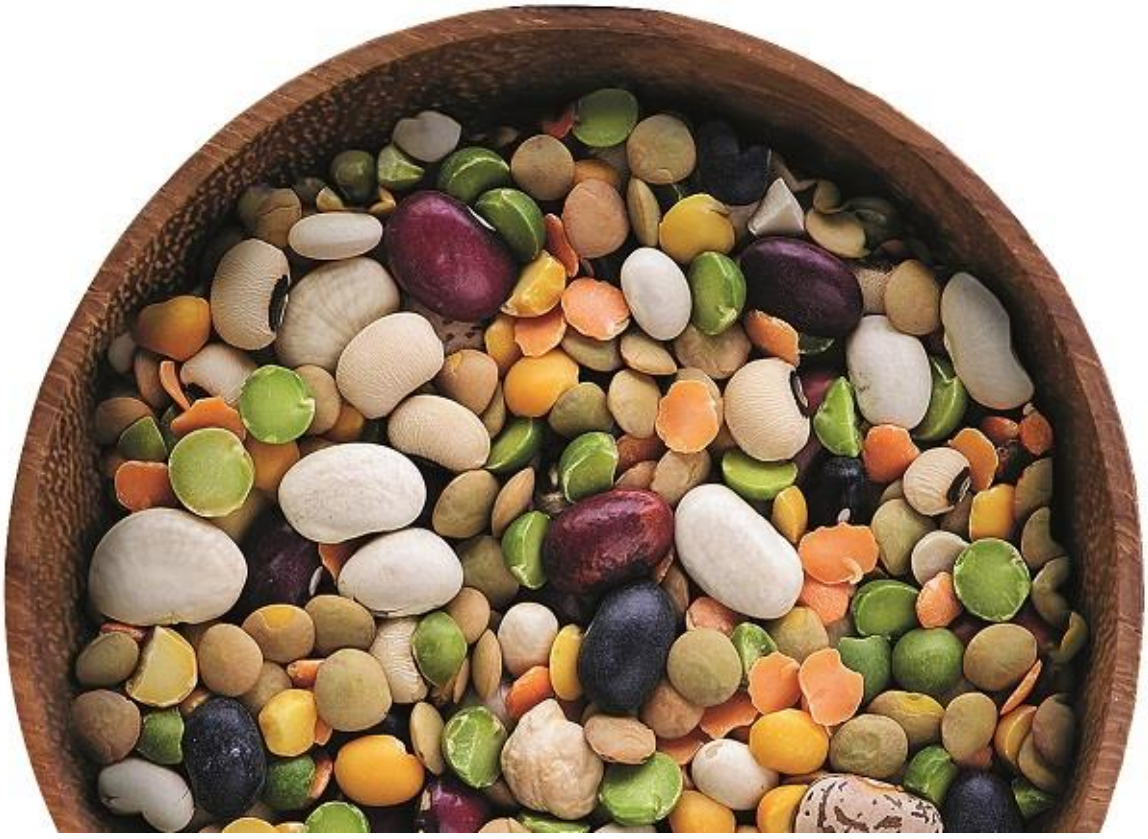
# Bradford Protein Assay

**Date**: September, 30th 2018



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# Content



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# 1 Summary Information

## 1.1 Partner Summary

| | |
|---|---|
| **SOP Code** | EU_TRUE_SOP_015 |
| **TRUE Partner Acronym** | AUA |
| **Primary Author** | Ntatsi, Georgia (*ntatsi@aua.gr*) |
| **Other Authors** | Savvas, Dimitrios |
| **Linked Reference and Hyperlink (if available)** | M.M. Bradford, 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein–dye binding. Anal. Biochem., 72, pp. 248-254 *https://doi.org/10.1016/0003-2697(76)90527-3* |
| **Associated files to use with the SOP [and function]** | NA |

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## 1.2 SOP Summary

### Title

**Bradford protein assay**

### Brief description

Protein concentration is determined using a Coomassie brilliant blue with bovine serum albumin as the standard (Bradford, 1976). The assay is based on the observation that the absorbance maximum for an acidic solution of Coomassie Brilliant Blue G-250 shifts from 465 nm to 595 nm when binding to protein occurs. Both hydrophobic and ionic interactions stabilise the anionic form of the dye, causing a visible color change.

The assay is useful since the extinction coefficient of a dye-albumin complex solution is constant over a 10-fold concentration range

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# 2 Protocol Steps

## Equipment

In addition to standard liquid handling supplies a visible light spectrophotometer is needed, with maximum transmission in the region of 595 nm, on the border of the visible spectrum (no special lamp or filter usually needed). Glass or polystyrene (cheap) cuvettes may be used, however the color reagent stains both. Disposable cuvettes are recommended (0.1 cm).

## Extraction Process

0.1 g of each pulverized, frozen sample (leaf and root samples) was homogenised with ice-cold **25 mM HEPES buffer (pH 7.8)**. The HEPES buffer contained **0.2 mM EDTA, 2 mM ascorbate and 2 % (w/v) polyvinylpyrolidon (PVP)**. The homogenates were centrifuged at 4 °C and 14,000 rpm. The supernatants obtained were used for enzyme analysis. The procedure for enzyme extraction was carried out at 0-4 °C.

**Reagents**

To prepare **1 L** of the necessary reagents, the following equation was used:

$$m = C * V * M \qquad (equation\ 1)$$

with m = weight (g)
C = molarity (mol/L)
V = volume to be prepared (L)
MB = molecular weight (g/mol)

- **25 mM HEPES buffer (pH 7.8)**

  Weigh 5.9575 g of HEPES (MB = 238.30) in a 1 L volumetric flask and adjust to 1 L with $H_2O$ (m = 0.025 * 1 * 238.30). Adjust the pH to 7.8 using either a diluted solution of HCl or NaOH

- **0.2 mM EDTA**

  Weigh 0.0745 g of EDTA (MB = 372.24) in a 1 L volumetric flask and adjust to 1 L with $H_2O$ (m = 0.0002 * 1 * 372.24).

- **2 mM ascorbate (AsA)**

  Weigh 0.352 g of Ascorbate (MB = 176.12) in a 1 L volumetric flask and adjust to 1 L with $H_2O$ (m = 0.002 * 1 * 176.12).

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- **2% (W/V) polyvinylpyrolidon PVP**
  - 2 g in 100 mL

Protein content is determined according to Bradford's method (Bradford, 1976), which utilises bovine serum albumin as standard. **Bradford Mix.**

## Reagents

- **Bradford reagent**

Dissolve 100 mg Coomassie Brilliant Blue G-250 in 50 mL 95% ethanol, add 100 mL 85% (w/v) phosphoric acid. Dilute to 1 liter when the dye has completely dissolved, and filter through Whatman #1 paper just before use.

The Bradford reagent should be a light brown in color. Filtration may have to be repeated to remove of blue components from the reagent. The Bio-Rad concentrate is expensive, but more sensitive than Homemade" reagents.

- (Optional) **1 M NaOH** (to be used if samples are not readily soluble in the color reagent).

Dissolve 40 grams of NaOH into a minimun of water to ensure complete solubility (m = 1 * 1 * 40). Then add $H_2O$ until the volume of the solution is 1 L. See also:
*http://www.nuigalway.ie/nanoscale/stars.htmL*

## Assay

- Warm up the spectrophotometer before use
- Aliquot 5 µL of the supernatant into the wells of a microplate
- Add 200 µL Bradford Mix (5 x diluted) to the samples. Do this quickly and measure immediately
- Add 5 mL dye reagent and incubate for 5 min
- Measure the absorption at 595 nm and relate to Standard curve prepared with BSA in NaOH (0, 0.05, 0.1, 0.2 … to 1 mg/mL).

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## Calculation Process

First from Linear Regression between the Standard Values and the Absorbances:

$$\mathbf{Y = AX + B} \text{ and therefore } \mathbf{X = (Y-B) / A}$$

with Y = Absorbance
X = Concentration (mmol/mL)
A = Slope
B = Intercept
R-square = 98-99%

Hence:

$$\mathbf{C_{final}\ (mg/g) = X\ (mmol/mL) / FW(g)}$$

with FW = Fresh Weight

## Comments

The dye reagent reacts primarily with arginine residues and less so with histidine, lysine, tyrosine, tryptophan, and phenylalanine residues. As expected, the assay is less accurate for basic or acidic proteins. The Bradford assay is rather sensitive to bovine serum albumin, more so than "average" proteins, by about a factor of two. Immunoglogin G (IgG - gamma globulin) is the preferred protein standard. The addition of 1 M NaOH was suggested by Stoscheck (1990) to allow the solubilisation of membrane proteins and reduce the protein-to-protein variation in color yield.

Standard curve prepared with BSA in NaOH (0, 0.05, 0.1, 0.2 …..to 1 mg/mL). See how to set up an assay for suggestions as to preparing the standards.

Stock solution preparation: C = 1 mg/mL (10 mg BSA into 10 mL $H_2O$ = BSA stock solution).

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| Concentration (1 mL eppi) (µg/mL) | BSA stock solution (µL) | $H_2O$ (µL) |
|---|---|---|
| 1,000 | 1,000 | 0 |
| 900 | 900 | 100 |
| 800 | 800 | 200 |
| 700 | 700 | 300 |
| 500 | 500 | 500 |
| 100 | 100 | 900 |
| 50 | 50 | 950 |
| 20 | 20 | 980 |
| 0 | 0 | 1,000 |

## Additional References

Bradford, MM. A rapid and sensitive for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical Biochemistry 72: 248-254. 1976.

Stoscheck, CM. Quantitation of Protein. Methods in Enzymology 182: 50-69 (1990).

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## 3 Linked SOPs

| SOP Code | SOP Function |
|---|---|
| NA | NA |

## 4 Disclaimer

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## 6 Citation

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