Deep Science Publishing
https://doi.org/10.70593/978-93-49307-52-0_33

Chapter 33

Lowry method for protein quantification: Principles, protocols, and applications in biochemical analysis

Keshawanand Tripathi[1*], Yashdeep Srivastava[1], Narendra Kumar[3]
[1]*Department of Biotechnology, Invertis University, Bareilly, Uttar Pradesh, India.*
[3]*School of Biotechnology and Bioengineering, Institute of Advanced Research, Gandhinagar, Gujarat, India.*
[*]Email: tripathikn009@gmail.com

1. Introduction:

The Lowry method, developed by Oliver H. Lowry and colleagues in 1951, is a classic and widely utilized technique for the quantification of protein concentration in biological samples. This assay is based on the principle of colorimetric detection of protein-bound copper ions resulting from the reduction of Folin-Ciocalteu reagent under alkaline conditions (Lowry et al., 1951). The assay offers high sensitivity and a broad dynamic range, making it suitable for both routine laboratory analyses and research applications (Kruger). By measuring the absorbance of the resulting chromogenic complex at 750 nm, the concentration of proteins in samples can be accurately determined (Hartree, 1972; Tripathi et al., 2013 a,b). Despite its effectiveness, the Lowry method requires meticulous attention to detail in sample preparation and handling due to its sensitivity to interfering substances. Overall, the Lowry method remains a cornerstone in protein quantification, providing researchers with a reliable and versatile tool for protein analysis in diverse biological contexts (Wilson, and Walker, 2010; Kruger 2009).

2. Materials Required:

1. Protein samples
2. Standard protein solutions (e.g., Bovine Serum Albumin - BSA)
3. 1N Folin-Ciocalteu reagent
4. Copper sulfate solution (0.5%)
5. Sodium carbonate solution (5%)
6. 1N Sodium hydroxide
7. Sodium potassium tartarate (1%)
8. Microplate or cuvette
9. Spectrophotometer capable of measuring absorbance at 750 nm
10. Microcentrifuge tubes
11. Distilled water
12. Micropipettes and tips

3. Procedure:

1. Make the standard reagents according to the table below:

Reagent/Standard	Composition
5% Na_2CO_3	in 0.1 N NaOH
1% NaK Tartrate	in H_2O
0.5% $CuSO_4$.5 H2O	in H_2O
Reagent I	48 ml of A, 1 ml of B, 1 ml C
Reagent II	1-part Folin-Phenol [2 N]: 1 part water
BSA Standard	1 mg/ml

2. To make BSA working standard solutions, add 0.2 ml of BSA to the five test tubes and then top each with 1 ml of distilled water.
3. Make a blank reference using a single test tube holding just one ml of distilled water.
4. To each tube add 4.5 ml of Reagent I, then incubate for ten minutes.
5. After the first half hour, give each tube 0.5 ml of reagent II and let it to incubate for a further half hour.
6. To build a standard curve, measure each solution's absorbance at 660 nm. Find the protein concentration in the provided sample using the standard curve.

S.No	Vol. of BSA (ml)	Conc .of BSA (mg/ ml)	Vol. of Distilled water (ml)	Vol. of Reag ent I (m l)	Incuba -tion for 10 min	Vol. of Reagent II (ml)	Incubation for 30 min	OD at 660 nm
1								
2								
3								
4								
5								

4. Precautions:

1. Ensure all spectrophotometric equipment is calibrated and maintained according to manufacturer specifications.
2. Use high-purity chemicals and reagents to prepare Lowry reagent and protein samples.
3. Handle protein samples carefully to avoid contamination and denaturation.
4. Mix Lowry reagent and protein samples thoroughly but gently to ensure uniform reaction.
5. Incubate samples at the appropriate temperature and duration to allow sufficient color development.

6. Perform blank measurements using the Lowry reagent alone to correct for background absorbance.
7. Use a spectrophotometer to measure absorbance at the specific wavelength recommended for the Lowry assay.
8. Prepare a standard curve using known concentrations of protein standards to correlate absorbance with protein concentration.
9. Ensure protein standards cover the expected range of concentrations in your samples for accurate estimation.
10. Validate the linearity of the standard curve to ensure the assay adheres to the principles of the Lowry method.
11. Perform protein estimation in triplicate or more to ensure reproducibility and reliability of results.
12. Dispose of used reagents, protein samples, and contaminated materials according to laboratory waste disposal guidelines.

5. References:

1. Wilson, K., & Walker, J. (2010). *Principles and Techniques of Biochemistry and Molecular Biology* (7th ed.). Cambridge University Press.
2. Lowry, O.H., Rosebrough, N.J., Farr, A.L., & Randall, R.J. (1951). Protein measurement with the Folin phenol reagent. *Journal of Biological Chemistry, 193*(1), 265-275.
3. Hartree, E.F. (1972). Determination of protein: A modification of the Lowry method that gives a linear photometric response. *Analytical Biochemistry, 48*(2), 422-427.
4. Kruger, N.J. (2009). The Lowry method for protein quantitation. *The Protein Protocols Handbook, 17*(1), 9-15.
5. Tripathi, K., Sharma, N. K., Rai, V., & Rai, A. K. (2013). Low cellular P-quota and poor metabolic adaptations of the freshwater cyanobacterium *Anabaena fertilissima* Rao during Pi-limitation. *Antonie van Leeuwenhoek, 103*, 277–291.
6. Tripathi, K., Sharma, N. K., Kageyama, H., Takabe, T., & Rai, A. K. (2013). Physiological, biochemical, and molecular responses of the halophilic cyanobacterium *Aphanothece halophytica* to Pi-deficiency. *European Journal of Phycology, 48*(4), 461–473.