

# assays: protein

## The Lowry Method

The Lowry method for determining protein concentration is essentially a biuret reaction that incorporates the use of Folin-Ciocalteu reagent for enhanced color development. The Lowry procedure is more commonly used in research applications because it is ten times more sensitive than the biuret reaction. In the Lowry method, protein is first treated with alkaline copper sulfate in the presence of tartrate. This "incubation" is then followed by addition of the Folin-phenol reagent. It is believed that the enhancement of the color reaction in the Lowry procedure occurs when the tetradentate copper complexes transfer electrons to the phospho-molybdic/phosphotungstic acid complex ( $\text{Mo}^{6+}/\text{W}^{6+}$ , Folin phenol reagent). Reduction of the Folin phenol reagent yields a blue color read at 750 nm.<sup>17,18</sup>

Although the Lowry method has the distinction of being the most referenced assay in the biochemical literature and has become the standard for protein quantitation, it is also well known for its deficiencies. For example, the alkaline copper reagent is unstable and requires daily preparation with a multi-step procedure that is time- and labor-intensive. In addition, the assay has been shown to be photosensitive. As a practical matter, precautions

should be taken to subject the samples to the same level of illumination during the procedure.<sup>26</sup>

Several modifications of the original Lowry procedure have been reported. For example, modifications have been made to simplify the procedure<sup>4,5</sup> and to improve the following: linearity of response;<sup>6</sup> reproducibility and sensitivity;<sup>23</sup> stability and chemistry of color development; stability of the biuret reagent;<sup>2,5</sup> and speed.<sup>27</sup> Other modifications have dealt with interfering substances<sup>7</sup> and approaches to the analysis of protein samples in the presence of biomaterials such as lipids.

Much has been published regarding substances that interfere with protein determination using the Lowry procedure. Compounds commonly known to interfere with the Lowry assay include: detergents and carbohydrates;<sup>8,19</sup> glycerol, Tricine and EDTA;<sup>20</sup> Tris;<sup>21</sup> potassium and sulfhydryl and disulfide containing compounds;<sup>9</sup> magnesium;<sup>21</sup> and calcium.<sup>22</sup>

The addition of oxalate to samples undergoing estimation using the Lowry assay has been shown to substantially reduce the errors associated with  $\text{Ca}^{++}$  interference. Pre-treatment of samples with sodium oxalate reduces errors by 70-95%.<sup>22</sup>

As a result of Pierce modifications, the classic Lowry method is now easier than ever to perform.

The Pierce Modified Lowry Protein Assay Reagent (Product #23240) offers the following benefits:

- Eliminates the need to prepare fresh reagent
- Replaces the original Lowry with a super-stabilized formulation that yields results with 100% correlation to the original Lowry assay
- Remains stable for 1 year

The Pierce Modified Lowry Protein Assay Reagent is ideal for loyal Lowry users who would like the increased convenience of a stable, pre-formulated product.

## References

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