

## Product Information

### Folin & Ciocalteu's phenol reagent

suitable for determination of total protein by Lowry method

Catalog Number **F9252**

Store at Room Temperature

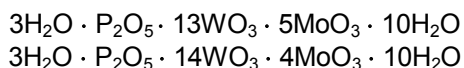
### Product Description

Appearance: Clear, bright yellow solution.

Acid concentration: 1.9–2.1 N based on sodium hydroxide titration.

A procedure for preparation of the Folin & Ciocalteu's phenol reagent has been published.<sup>1</sup> Dissolve 10 g of sodium tungstate and 2.5 g of sodium molybdate in 70 ml of water. Add 5 ml of 85% phosphoric acid and 10 ml of concentrated hydrochloric acid. Reflux for 10 hours. Add 15 g of lithium sulfate, 5 ml of water, and 1 drop of bromine. Reflux for 15 minutes. Cool to room temperature and bring to 100 ml with water.<sup>1</sup>

Hexavalent phosphomolybdic/phosphotungstic acid complexes with the following structures are formed in solution:<sup>2</sup>



Folin & Ciocalteu's phenol reagent does not contain phenol. Rather, the reagent will react with phenols and non-phenolic reducing substances to form chromogens that can be detected spectrophotometrically. It can also be used as a spray reagent in chromatographic procedures. The color development is due to the transfer of electrons at basic pH to reduce the phosphomolybdic/phosphotungstic acid complexes to form chromogens in which the metals have lower valence.<sup>3</sup>

The most common usage of this reagent is in the Lowry method for determining protein concentration.<sup>4</sup> In this method, protein is pretreated with copper(II) in a modified biuret reagent (alkaline copper solution stabilized with sodium potassium tartrate). Addition of Folin & Ciocalteu's phenol reagent generates chromogens that give increasing absorbance between 550–750 nm. Normally, absorbance at the peak (750 nm) or shoulder (660 nm) are used to quantitate protein concentrations between 1–100 µg/ml while absorbance at 550 nm is used to quantitate higher protein concentrations.

In the absence of copper, color intensity would be determined primarily by the tyrosine and tryptophan content of the protein, and to a lesser extent by cysteine and histidine. Copper(II) enhances color formation by chelation with the peptide backbone, thus facilitating the transfer of electrons to the chromogens. Copper(II) has no effect on color formation by tyrosine, tryptophan, or histidine, but reduces that due to cysteine.<sup>2-6</sup>

Many modifications of the original assay procedure have been published,<sup>2</sup> including methods for enhancing the color development,<sup>5,7</sup> for determining the content of insoluble proteins,<sup>4,8</sup> and for automating the procedure.<sup>9</sup> A list of compounds that interfere with the Lowry protein assay, including many buffers, chelating agents, detergents, and cyclic organic compounds, has been published.<sup>2</sup> To control for the effect of these compounds on color development and, thus, on the calculated protein concentration of the sample, it is essential that the blank and standards be made up in the same medium as the samples.

Two kits for quantitating total protein based on the Lowry procedure are available from Sigma. One kit (Catalog Number TP0200) uses a micro-Lowry method incorporating the modifications of Onishi and Barr to increase assay sensitivity.<sup>5</sup> Another kit (Catalog Number TP0300) employs Peterson's modifications of the micro-Lowry method to facilitate the dissolution of relatively insoluble proteins.<sup>8</sup>

### Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

### Preparation Instructions

The reagent can be diluted with water.

### Storage/Stability

Folin & Ciocalteu's phenol reagent should be stored tightly capped at room temperature. The product remains active for at least 4 years. If the solution acquires a greenish tint, it should be discarded.

### References

1. Krebs, K.G., et al., in *Thin Layer Chromatography*, Stahl, E., (ed.), Springer-Verlag, (New York, NY: 1969) p. 878.
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4. Lowry, O.H., et al., *J. Biol. Chem.*, **193**, 265-275 (1951).
5. Onishi, S.T., and Barr, J.K., *Anal. Biochem.*, **86**, 193-200 (1978).
6. Chattopadhyay, M.K.J., *Pharm. Pharmacol.*, **45**, 80 (1993).
7. Larson, E., et al., *Anal. Biochem.*, **155**, 243-248 (1986).
8. Peterson, G.L., *Anal. Biochem.*, **83**, 346-356 (1977).
9. Fryer, H.J.L., et al., *Anal. Biochem.*, **153**, 262-266 (1986).

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