

47641 Folin-Ciocalteu's phenol reagent

Product Description:

Appearance: Yellow greenish solution.

Acid concentration: 2 M based on sodium hydroxide titration.

Folin & Ciocalteu's phenol reagent should be stored tightly capped at room temperature. The reagent can be diluted with deionized water.

Method of Preparation:

Dissolve 10 g sodium tungstate and 2.5 g sodium molybdate in 70 ml water. Add 5 ml 85% phosphoric acid and 10 ml concentrated hydrochloric acid. Reflux for 10 hr. Add 15 g lithium sulfate, 5 ml water and 1 drop bromine. Reflux for 15 min. Cool to room temperature and bring to 100 ml with water. 1 Hexavalent phosphomolybdic/phosphotungstic acid complexes with the following structures are formed in solution.²



Applications:

Folin & Ciocalteu's phenol reagent does not contain phenol. Rather, the reagent will react with phenols and nonphenolic 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.³

The most common usage of this reagent is in the Lowry method for determining protein concentration.⁴ 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 nm and 750 nm. Normally, absorbance at the peak (750 nm) or shoulder (660 nm) are used to quantify 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.^{2,4,5,6}

Many modifications of the original assay procedure have been published² including methods for enhancing the color development,^{5,7} for determining the content of insoluble proteins,^{4,8} and for automating the procedure.⁹ A list of compounds that interfere with the Lowry protein assay, including many buffers, chelating agents, detergents, and cyclic organic compounds, has been published.² 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.



References

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Precautions and Disclaimer:

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