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ProductInformation

Total Protein Kit, Micro Lowry, Peterson's Modification

Product Codes **TP0300 and L 3540**

TECHNICAL BULLETIN

Product Description
The method of Lowry has been for decades the procedure of choice for quantitation of soluble proteins due to its sensitivity, simplicity, and precision. The procedure described here is based on Peterson's modification of the micro Lowry method and utilizes sodium dodecylsulfate, included in the Lowry Reagent, to facilitate the dissolution of relatively insoluble lipoproteins.[1]

For many proteins, the Lowry reaction can be run directly on the protein solution. However, interference in the direct Lowry procedure is commonly caused by other chemicals in the protein solution, such as tris, ammonium sulfate, EDTA, sucrose, citrate, amino acid and peptide buffers, and phenols. The procedure with protein precipitation, which uses DOC (deoxycholate) and TCA (trichloroacetic acid), eliminates all these interferences with the exception of phenols. However, the amount of various proteins recovered through the precipitation step may vary depending on the particular proteins assayed.

The procedure is based on two chemical reactions. The first is the biuret reaction, in which the alkaline cupric tartrate reagent complexes with the peptide bonds of the protein. This is followed by the reduction of the Folin & Ciocalteu's phenol reagent, which yields a purple color. Absorbance of the colored solution is read at a suitable wavelength between 500 nm and 800 nm. The protein concentration is determined from a calibration curve.

Components

Lowry Reagent, Powder 5 x 2 g
Product Code L 3540

0.15% Deoxycholate (DOC) Solution 20 ml
Product Code D 8566
A 1.5 mg/ml aqueous solution of sodium deoxycholate

Trichloroacetic Acid (TCA) Solution, 20 ml
Product Code T 2574
A 72% (w/v) aqueous solution of trichloroacetic acid

Folin & Ciocalteu's Phenol Reagent, 1 btl
Product Code F 9252

Protein Standard, 5 x 1 vial
Product Code P 5619
2 mg of BSA per vial

Precautions and Disclaimer
This product is for laboratory research use only, not for drug, household, *in vitro* diagnostic, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions
The Lowry Reagent Solution is prepared by adding 40 ml of water to a bottle of Lowry Reagent, Powder (Product Code L 3540). Mix well by inverting to completely dissolve the contents. Do not shake so as to minimize foaming.

The Folin & Ciocalteu's Phenol Reagent Working Solution is prepared by transferring the Folin & Ciocalteu's Phenol Regent (18 ml) to the amber glass bottle provided for the Working Solution. Rinse the Folin & Ciocalteu's Phenol Reagent bottle with 10 ml of water and add the rinse solution to the Working Solution bottle. Add an additional 80 ml of water to the Working Solution bottle and mix well. Store the Folin & Ciocalteu's Phenol Reagent Working Solution at room temperature.

A Protein Standard Solution (400 µg/ml) is prepared by adding an appropriate volume of water (approximately 5 ml) to the vial. The exact protein content of the Protein Standard vial may be found on a lot-specific Certificate of Analysis. Swirl gently to completely dissolve the contents.

Storage/Stability
Store the Lowry Reagent Solution and the Folin & Ciocalteu's Phenol Reagent at room temperature. Do Not Refrigerate.

Store the Protein Standard solution in a refrigerator (2-8 °C) or freezer (below 0 °C). The solution is stable for at least 3 months when stored refrigerated. Discard the Protein Standard Solution if turbidity develops.

Procedure
A. Protein Determination without Protein Precipitation
1. Prepare Standard Tubes by diluting the 400 µg/ml Protein Standard Solution in water to a volume of 1.0 ml in appropriately labeled test tubes:

Protein Standard Solution (ml)	**Water** (ml)	**Protein Concentration** (µg/ml)
0.125	0.875	50
0.250	0.750	100
0.500	0.500	200
0.750	0.250	300
1.000	0	400

2. Label a test tube Blank and add 1.0 ml of water.
3. Add the sample to the appropriately labeled test tube and dilute to 1.0 ml with water.
4. Add 1.0 ml of the Lowry Reagent Solution to Standard, Blank, and Sample tubes. Mix well.
5. Allow solutions to stand at room temperature for 20 minutes.
6. With rapid and immediate mixing, add 0.5 ml of the Folin & Ciocalteu's Phenol Reagent Working Solution to each tube.
7. Allow color to develop for 30 minutes.
   Note: If your cuvet requires more than a 2.5 ml volume, add an appropriate volume of water to each tube before reading. Treat Standard, Blank, and Sample tubes identically.
8. Transfer solutions to cuvets and measure the absorbance of the Standards and Sample tubes versus the Blank at a wavelength between 500 and 800 nm. Complete the absorbance readings within 30 minutes.
   Note: The amount of color varies with different proteins.[3]
9. Plot the absorbance values of the Standards versus their corresponding protein concentrations to prepare a calibration curve.
10. Determine the protein concentration of the Sample tube from the calibration curve. Multiply the results by the appropriate dilution factor to obtain the protein concentration in the original sample.

B. Protein Determination with Protein Precipitation
Note: Under the test conditions, some proteins are incompletely precipitated by trichloroacetic acid, thus resulting in lower values. It may be possible to compensate for partial loss during the precipitation step by using as the standard the same protein or one similar to that being assayed. In any case, the possibility of incomplete precipitation should be considered.
1. Prepare Standard Tubes by diluting the 400 µg/ml Protein Standard Solution in water to a volume of 1.0 ml in appropriately labeled microcentrifuge tubes:

Protein Standard Solution (ml)	**Water** (ml)	**Protein Concentration** (µg/ml)
0.125	0.875	50
0.250	0.750	100
0.500	0.500	200
0.750	0.250	300
1.000	0	400

2. Label a plastic microcentrifuge tube Blank and add 1.0 ml of water.
3. Add sample to the appropriately labeled plastic microcentrifuge tube and dilute to 1.0 ml with water.
   Note: To eliminate ampholyte interference, add sodium chloride to a concentration of 0.1 M before the DOC/TCA precipitation.
4. Add 0.1 ml of the DOC Solution to each Standard, Blank, and Sample tube. Mix well and allow to stand at room temperature for 10 minutes.
5. Add 0.1 ml of the TCA Solution to each Standard, Blank, and Sample tube and mix well.
6. Centrifuge the solutions for 5 to 10 minutes to pellet the precipitates.
7. Decant and blot away the supernatants.
8. Dissolve the pellets in 1.0 ml of the Lowry Reagent Solution and transfer the solutions to appropriately labeled test tubes.
9. Rinse the microcentrifuge tubes with 1.0 ml of water and quantitatively add the rinse solutions to their respectively labeled test tubes. Mix well.
10. Allow solutions to stand at room temperature for 20 minutes.
11. With rapid and immediate mixing, add 0.5 ml of the Folin & Ciocalteu's Phenol Reagent Working Solution to each tube.
12. Allow color to develop for 30 minutes.
    Note: If your cuvet requires more than a 2.5 ml volume, add an appropriate volume of water to each tube before reading. Treat Standard, Blank, and Sample tubes identically.

13. Transfer solutions to cuvets and measure the absorbance of the Standards and Sample tubes versus the Blank at a wavelength between 500 and 800 nm. Complete the absorbance readings within 30 minutes.
    Note: The amount of color varies with different proteins.[3]
14. Plot the absorbance values of the Standards versus their corresponding protein concentrations to prepare a calibration curve.
15. Determine the protein concentration of the Sample tube from the calibration curve. Multiply the results by the appropriate dilution factor to obtain the protein concentration in the original sample.

References

1. Peterson, G.L., Analyt. Biochem., **83**, 346 (1977).
2. Bensadoun, A., and Weinstein, D., Analyt. Biochem., **70**, 241 (1976).
3. Lowry, O.H. et al., J. Biol. Chem., **193**, 265 (1951).

LCM/GL/MAM 10/03

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