



Product Information

Total Protein Kit, Micro Lowry, Onishi & Barr Modification

Product Codes **TP0200** and **B 3934**

Storage Temperature 2–8 °C

TECHNICAL BULLETIN

Product Description

The biuret and Lowry¹ procedures are methods for protein determination. The former is widely used for clinical assays. The latter, though more sensitive, is used for investigative work and is limited by poor stability of combined reagents, non-reproducibility of color, especially at low protein concentration, and non-linear chromogenic response with protein concentration.

Ohnishi and Barr² modified the biuret reagent for the Lowry procedure, thereby simplifying it, while improving the stability of the combined reagent. The procedure described here is a modification of their method. Since the method is very sensitive, the sample is diluted to a final protein concentration range of 150 - 1,000 µg/ml. The sample volume required has been reduced and vortex mixing has been eliminated. A protein sample is mixed with the Biuret Reagent and later with Folin and Ciocalteu's Phenol Reagent. The absorbance is read at a suitable wavelength between 550 and 750 nm (maximum color is observed at 700–750 nm). Protein concentrations are determined from a calibration curve.

Components

Sufficient reagents are supplied for 50 assays including reactions for standards and blanks.

Biuret Reagent 110 ml
(Product Code B 3934)
Reagent contains 0.75 mM cupric sulfate and 94 mM sodium hydroxide. It also contains tartrate, iodide, and carbonate.

Folin and Ciocalteu's Phenol Reagent 5 ml
(Product Code F 6678)
2.0 Normal

Protein Standard 5 ml
(Product Code P 9369)

The standard contains 100 mg/ml of bovine serum albumin in 0.85% sodium chloride solution with 0.05% sodium azide added as preservative.

Reagents and Equipment Required but Not Provided

- spectrophotometer to determine absorbance in the range of 550–750 nm
- Cuvets
- Pipettes
- Test Tubes
- 50 ml volumetric flask
- 0.85% Sodium Chloride Solution (Product Code S 0817)

Note: This solution may be prepared by dissolving 8.5 g of sodium chloride (Product Code S 9625) in 1,000 ml of water.

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 supplied reagents are ready to use.

Storage/Stability

It is recommended to store the kit at 2–8 °C. Store the Folin and Ciocalteu's Phenol Reagent at room temperature. Store the prepared 0.85% Sodium Chloride Solution at room temperature.

The Biuret Reagent is suitable for use in the absence of precipitation.

Procedure

All glassware must be free of protein. This assay is dependent on the tryptophan and tyrosine content of proteins. Therefore, the presence of either of these as free amino acid contaminants will interfere with assay results. Glycine decreases the color developed with protein by up to fifty percent. Ammonium sulfate at a final concentration greater than 0.15% decreases color development.² Most phenols, except nitrophenol, reduce the reagent and some color change may occur. Uric acid, guanine, and xanthine react with the phenol reagent, whereas guanosine does not react appreciably.² The procedure also is affected by potassium ions,³ magnesium ions,⁴ EDTA,⁷ thiol reagents,³ tris,⁴ and some carbohydrates.⁵⁻⁷

Note: This procedure becomes insensitive above a protein concentration of 1,000 µg/ml. Protein concentrations above this level can be assayed by diluting the sample appropriately with 0.85% Sodium Chloride Solution.

1. Dilute the test samples with 0.85% Sodium Chloride Solution to give a final protein concentration range of 150 - 1,000 µg/ml.
2. Label a test tube for the Blank and one for each test sample (Test 1 and Test 2).
3. To the test tube labeled Blank, add 0.2 ml of 0.85% Sodium Chloride Solution.
4. Add 0.2 ml of a diluted test sample solution prepared in step 1 to the appropriately labeled test tube.
5. To each test tube add 2.2 ml of the Biuret Reagent (Product Code B 3934). Mix well and allow to stand at room temperature for 10 minutes.
6. Add 0.1 ml of the Folin and Ciocalteu's Phenol Reagent (Product Code F 6678) to each tube. Mix each tube well immediately after addition. Allow to stand at room temperature for 30 minutes.
7. Transfer the contents of the test tubes to appropriate cuvetts and read absorbance using the Blank as reference at the same wavelength and on the same instrument used to prepare the calibration curve. Complete readings within 30 minutes.
8. Determine the protein concentration (µg/ml) of each diluted test sample from the calibration curve. Multiply by the dilution factor (step 1) to obtain protein concentration in each test sample.

Calibration Curve

1. Pipette 0.5 ml of the Protein Standard (Product Code P 9369) into a 50 ml volumetric flask. Dilute to 50 ml with 0.85% Sodium Chloride Solution.
2. Pipette into 5 test tubes the solutions indicated in Table 1.

Table 1.
Solutions for Calibration Curve

Test Tube	Diluted Protein Standard (step 1) (ml)	0.85% Sodium Chloride Solution (ml)	Protein (µg/ml)
1	0	0.20	0
2	0.05	0.15	250
3	0.10	0.10	500
4	0.15	0.05	750
5	0.20	0	1,000

3. To each test tube add 2.2 ml of the Biuret Reagent (Product Code B 3934). Mix well and allow to stand at room temperature for 10 minutes.
4. Add 0.1 ml of the Folin and Ciocalteu's Phenol Reagent (Product Code F 6678) to each tube. Mix each tube well immediately after addition. Allow to stand at room temperature for 30 minutes.
5. Transfer the contents of the test tubes to appropriate cuvetts and read absorbance at 550–750 nm (maximum color at 700–750 nm) using test tube 1 as reference.
6. A calibration curve is obtained by plotting the absorbance values versus protein concentration. A curved line passing through the origin should be obtained. Alternatively, the absorbance values can be plotted versus protein concentration on logarithmic paper (2 cycle). A straight line should be obtained.

References

1. Lowry, O.H., et al., Protein measurement with the Folin phenol reagent. *J. Biol. Chem.*, **193**, 265 (1951).
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3. Vallejo, C.G., and Lagunas, R., Interferences by sulfhydryl, disulfide reagents and potassium ions on protein determination by Lowry's method. *Anal. Biochem.*, **36**, 207 (1970).
4. Kuno, H., and Kihara, H.K., Simple microassay of protein with membrane filter. *Nature*, **215**, 974 (1967).
5. Neurath, A.R., Interference of sodium ethylenediaminetetraacetate in determination of proteins and its elimination. *Experientia*, **22**, 290 (1966).
6. Lo, C.H., and Stelson, H., Interference by polysucrose in protein determination by the Lowry method. *Anal. Biochem.*, **45**, 331 (1972).
7. Peterson, G.L., Review of the Folin phenol protein quantitation method by Lowry, Rosenbrough, Farr and Randall. *Anal. Biochem.*, **100**, 201 (1979).

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