FT-1E5351

Biuret-Gornall Protein Assay

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Product Description

Cat.number: **1E5351**

Name: **Biuret-Gornall Protein Assay**

Contains 1L of Biuret-Gornall Reagent

Applications: Protein determination and quantitation for solution materials with >1mg/ml protein.

Store the at room temperature (stable for 2 years)(Z)

Associated product: # UPQ84171 BSA standard

Introduction

Protein determination is performed in many research areas. This protein assay reagent #1E5351 uses the Biuret method standardized by Gornall (1951) for the qualitative and quantitative determination of total proteins in solution of a variety of samples (biological matrices, fractions,…). It is a simple and rapid colorimetric method, read at 540 nm.
This assay is a good general protein assay for material available in medium to large quantity/concentration.
See related product for alternative assays of protein concentration for superior sensitivity, linearity or compatibility.

The Biuret reaction occurs under alkaline pH conditions with at least 2-4 peptidic bounds:

Copper ions + Proteins → Copper-Protein Complexes (Purple)

The formed complexes can be measured spectrophoto- metrically in the 540 nm region, supporting the peptidic nature.
The intensity of the color is proportional to the total protein concentration, make the assay quantitative.
The major drawback of the Biuret-Gornall assay relies on its relatively low detection sensitivity (20mg/ml). It however keep the advantage of giving the same signal independently from protein aminoacids composition, compared with alternative methods that have higher sensitivity (down 1mg/ml, and below by fluorescence). The protocol is also simple and rather rapid (20min). Finally, the method is well established unlike some others.

Our Biuret-Gornall reagent is supplied as a ready-to-use solution.

Directions for use

■ Reagents and Equipment required but not provided

- Spectrophotometer measuring absorbance at 540 nm
- Cuvettes suitable for use at 540 nm
- Pipettes
- Timer
- Protein Standard (e.g. BSA standard #UP36859A)

■ Samples and Standards

Check for the buffer composition of your samples (possible interfering substances – see below).
If this buffer is available, use it as a negative control (blank). It even can be used as the buffer for diluting standards and samples for best results.
The assay is linear to 160 mg/ml. It is not suitable for use if a precipitate forms.
Prepare dilutions of your standard from 0.5 to 160mg/ml with dezionized water (or any suitable buffer).
Dilute samples that are too much concentrate with the same dilution buffer (water). The result of these (diluted) Test-Samples should be and correct accordingly.

■ Assay Procedure

1. Prepare blank (assay buffer alone), Standards and Test Samples in labeled test tubes
Add 20 µl of deionized water, protein standard, or test samples into the appropriately labeled test tubes.

2. Pipette 1.0 ml of the Total Protein Reagent into each tube.
Mix by gentle inversion.

4. Incubate each tube for 20 minutes at ambient temperature.

5. Read and record the absorbance at 540 nm (OD or A_{540nm}) of all tubes versus the Reagent Blank as the reference.
Note: The biuret color is stable for at least 1 hour.

■ Results and calculations
Determine the total protein concentration (mg/ml) in the samples from net ODs using the standard curve

$$\text{Total Protein (mg/ml)} = \frac{A_{540nm}\ \text{(sample)}\ X\ \text{Concentration of Standard (mg/ml)}}{A_{540nm}\ \text{(standard)}}$$

Rem: An absorbance change of 0.06 corresponds to a protein concentration of approximately 10 mg/ml.
The assay is linear to 160 mg/ml.

■ Precautions
Use clean glassware (free of protein residues or film).
Avoid dust, fingerprints (used gloves).

■ Interfering substances
Ammonium salts[11] and the reducing agent dithiothreitol enhance the assay colour. Interference has also been observed with ammonium sulfate[6-8], glycerol[9], guanidine[10], Triton® X100 [10], Tween® 80 [10], and urea[8].
Biological materials known to interfere with the biuret method include lipids, bilirubin, hemoglobin, and dextran[4,5].
For removing interfering small substances, use the Protein Preparation Reagent Procedure (R5594A).

References

1. Grant, G.H., and Kachmer, J.F., Tietz, N.W., The proteins in body fluid. in Fundamentals of Clinical Chemistry. ed., W.B. Saunders (Philadelphia, PA: 1976), pp 358–374.
2. Cannon, D.C. et al., Proteins. in Clinical Chemistry - Principles and Technics, 2nd edition, Henry, R.J. et al., eds., Harper & Row (New York, NY: 1974), pp 422–431.
3. Doumas, B.T. et al., Clin. Chem., 27 , 1642 (1981).
4. Clinical Chemistry: Interpretation and Techniques, 2nd ed. Kaplan, A., and Szabo J., eds, 1983, p 157.
5. Crowley, L.V., Am. J. Clin. Pathol., 51 , 425 (1969).
6. Gornall, A.G. et al., J. Biol. Chem., 177 , 751-766 (1949).
7. de St. Groth, S.F. et al., Biochem. Biophys. Acta, 71 , 377-391 (1963).
8. Itzhak, R.F., and Gill, D.M., Anal. Biochem., 9 , 401-410 (1964).
9. Zishka, M.D., and Nishimura, J.S., Anal. Biochem., 34 , 291-297 (1970).
10. Futterman, S., and Rollins, M.H., Anal. Biochem., 51 , 443-447 (1973).
11. Layne, E. Methods in Enzymology 10 : 447-455, (1957).

Legals

imposed hazardous material charge – Inquire []

Disclaimers

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.
Triton is a trademark of Union Carbide Corporation.
Tween is a registered trademark of ICI Americas.

Related products and documents

●**Other Protein Assays**:

UP40840A BC Assay protein dosage kit

Suits most applications with superior linearity/working range, low protein/protein variations (2-3x less that Bradford), broad compatibility (detergents, nucleic acids, …). Based on the BCA method. Sensitivity: 0.5-5µgl/ml. 1-1500µg/ml working range.

UPF86400 CooAssay protein assay kit

Suits application needing simple and quick operating. Compatible with acids samples, reducers,…
Based on modified Bradford method. 562nm reading. Sensitivity 1µg/ml. 1-20 or 20-1500 g/ml working range.

CH4191 Protein&Peptide EpicoccoStab Fluorescent Assay Kit

Suits application needing simple and quick operating
Based on a synthetic analog of epicocconone dye. Sensitivity: 160ng/ml.

●**Protein Standard**

UP36859A BSA standard 2mg/ml
UPQ84171 BSA standard grade .
R5594A Protein Preparation Reagent●**Desalting tools**
CelluSep Dialysis tubing.

Ordering information

Catalog size quantities and prices may be found at http://www.interchim.com.

For any information, please ask : Uptima / Interchim; Hotline : +33(0)4 70 03 73 06

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