

Biuret Reagent

<u>Product Name</u>	<u>Product Code</u>	<u>Kit Packing</u>
Biuret Reagent	ML122-100ML	100 ml
	ML122-500 ML	500 ml

Introduction

There are a number of very good and sensitive protein assay methods available. One of the simplest and most common is the Biuret Protein Assay which is based on formation of colored coordination complexes in an alkaline solution between divalent copper ion and peptide bonds. The Biuret assay is considered as a general protein estimation method which involves a single incubation of 10 minutes.

Description

In Biuret reaction peptides containing three or more amino acid residues form a colored chelate complex with cupric ions (Cu^{2+}) in an alkaline environment containing sodium potassium tartrate. This became known as the biuret reaction because it is chemically similar a complex that forms with the organic compound biuret ($\text{NH}_2\text{-CO-NH-CO-NH}_2$) and the cupric ion. Biuret, a product of excess urea and heat, reacts with copper to form a light blue tetradentate complex. Single amino acids and dipeptides do not give the biuret reaction, but tripeptides and larger polypeptides or proteins will react to produce the light blue to violet complex that absorbs light at 540 nm. One cupric ion forms a colored coordination complex with four to six nearby peptide bonds. The intensity of the color produced is proportional to the number of peptide bonds participating in the reaction. Thus, the biuret reaction is the basis for a simple and rapid colorimetric reagent of the same name for quantitatively determining total protein concentration.

Application

Biuret reagent is mainly used for the quantitative estimation of protein samples by the Biuret assay method.

Composition

Biuret reagent consists of Copper sulphate and Sodium potassium tartrate.

Properties

Appearance	: Light blue colored solution
Clarity	: Clear and free of particles
Suitability test	: This solution has been tested and is suitable for use in quantitative estimation of protein samples by Biuret assay

Storage conditions

Biuret Reagent has to be stored at 2-8 °C. Under recommended condition, the reagent is stable for 12 months.

Quality control

Biuret Reagent is tested for quantitative estimation of unknown protein samples.

Estimation of unknown protein using standard curve (with BSA, HiMedia Product Code: MB083)

1. Take nine tubes and label them as Blank and 1 to 8.
2. Make dilutions of Protein (BSA) standards with concentrations of 10, 8, 6, 4, 2, 1 mg/200 μ l by transferring respective amount of BSA from the standard protein solution (50 mg/ml) and adjusting it to a total volume of 200 μ l by adding distilled water as mentioned in table 2.
3. Add 2 ml of Biuret reagent to each test tube including the Blank and Unknown tubes. Mix well.
4. Keep at room temperature for 10 minutes.
5. Switch on the Spectrophotometer, select the wavelength at 540 nm and let it warm before taking the absorbance (OD). First take the OD of Blank and make it zero.
6. Remove Blank tube and take the OD of all the tubes and record it. Wash the cuvette after taking OD of each sample.
7. Plot a Standard Curve of absorbance at 540 nm on "Y" axis versus concentration of protein mg/200 μ l on "X" axis.
8. Record the value "x" of Unknown from graph corresponding to the optical density reading of the test sample.

Note: If the samples have been diluted, multiply the concentration of the sample by dilution factor.

Tube No.	Blank	1	2	3	4	5	6	7	8
Conc. of BSA (mg)	0.0	1	2	4	6	8	10	Test sample 1	Test Sample 2
Amt of Stock (μ l)	0.0	20	40	80	120	160	200	200 μ l	200 μ l
Amt of Distilled Water (μ l)	200	180	160	120	80	40	0.0		
Amt of Biuret reagent (ml)	2	2	2	2	2	2	2	2	2
Keep at room temperature for 10 minutes									
Absorbance at 540 nm									

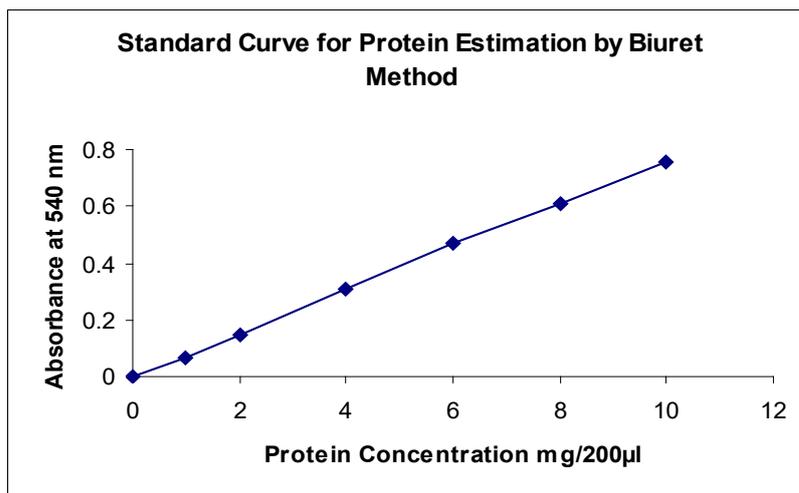


Fig: Standard curve for protein estimation by Biuret assay

Unknown protein concentration can be calculated using following formula:

$$\text{Protein Concentration in Test Sample} = \frac{\text{Concentration of Unknown in "mg"} \times 5}{\text{Volume of sample in "}\mu\text{l"}}$$

$$= \text{_____ mg/ml}$$

Warning and Precautions

Not for Medicinal Use. Read the SDS carefully before beginning the protocol. Wear protective gloves/protective clothing/eye protection/face protection. Follow good clinical laboratory practices while handling clinical samples. Standard precautions should be followed as per established guidelines. Safety guidelines may be referred in safety data sheets of the product.

Performance and Evaluation

Performance of the solution is expected when the solution is stored at recommended temperature and within the expiry period.

Safety Information

The Biuret Reagent is for laboratory use only, not for drug, household or other uses. Take appropriate laboratory safety measures and wear gloves and safety goggles when handling. Not compatible with disinfecting agents containing bleach. Please refer the Safety Data Sheet (SDS) for information regarding hazards and safe handling practices.

Technical Assistance:

At HiMedia we pride ourselves on the quality and availability of our technical support. For any kind of technical assistance, mail at mb@himedialabs.com.

Symbol

	Manufacturer		Do not use if package is damaged
	Batch code		Temperature limit
	Date of manufacture (YYYY-MM)		Consult instructions for use
	Use-by date (YYYY-MM)		Catalogue number

Identification No.: PIML122

Rev No.: 02

Date of Issue: 2025-11

Disclaimer :

User must ensure suitability of the product(s) in their application prior to use. Products conform solely to the information contained in this and other related HiMedia™ publications. The information contained in this publication is based on our research and development work and is to the best of our knowledge true and accurate. HiMedia™ Laboratories Pvt Ltd reserves the right to make changes to specifications and information related to the products at any time. Products are not intended for human or animal or therapeutic use but for laboratory, diagnostic, research or further manufacturing use only, unless otherwise specified. Statements contained herein should not be considered as a warranty of any kind, expressed or implied, and no liability is accepted for infringement of any patents.

HiMedia Laboratories Pvt. Ltd. Reg.office : Plot No. C-40, Road No. 21Y, MIDC, Wagle Industrial Estate, Thane, (West) 400604, Maharashtra, INDIA. Customer Care No.: 00-91-22-6116 9797 Tel: 00-91-22-6147 1919, 6903 4800 Email: techhelp@himedialabs.com Website: www.himedialabs.com