



TOTAL PROTEIN REAGENT
C254-01

Contents	Product No.	Package
Total Protein Reagent	C254-01	1 x 500 mL

REAGENT PREPARATION

This reagent is packaged ready for use.
No preparation is required.

REAGENT STORAGE AND STABILITY

Store unopened reagent preferentially between 12-26°C (Reagent can, however, be stored anywhere between 2-30°C)

When stored as directed, the reagent is stable until the expiration date stated on the label.

NOT FOR USE IN UNPROFESSIONAL SETTINGS

FOR TECHNICAL ASSISTANCE:

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TOTAL PROTEIN REAGENT C254-01 MANUAL/AUTOMATED APPLICATION

Intended Use

For **IN VITRO** quantitative determination of Total Protein in serum.

Clinical Significance

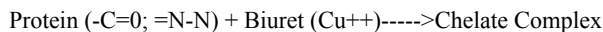
Measurements of Total Protein are primarily used for diagnosing dehydration, malignancies, cirrhosis, malnutrition, vitamin deficiencies, as well as for monitoring the causes and treatments. (1)

Method History

In 1946 Weichselbaum (2) proposed the use of the Biuret reaction for the determination of Total Protein in serum. The proposed method was modified by Kingsley who made the procedure simple and practical for routine use in the clinical laboratory. The Catachem Total Protein method is based upon Weichselbaum's Biuret formulation in which potassium tartrate is used as a coupling agent to keep the copper in solution and potassium iodide is used to prevent autoreduction.

Method Principle

A serum sample is mixed with the Biuret reagent. The carboxyl (-C=O) and imine (=N-N) groups of the peptide bond present in the protein molecular structure react with the copper ions under alkaline conditions to form a colored chelate complex. The intensity of the color produced is read at 550nm and it is directly proportional to the Total Protein concentration in the serum sample. The reaction scheme illustrates the reaction that occurs in this method:



Reagent Content

The concentrations of the active ingredients in the reagent are approximately as follows.

Biuret Reagent

One liter contains:

Cupric Sulfate .5H2O	12 mmol/L
Potassium Sodium Tartrate	50 mmol/L
Sodium Hydroxide	200 mmol/L
Potassium Iodide	30 mmol/L
Nonreactive ingredients and stabilizer	

Precautions

Avoid contact of reagent with skin and eyes. Should contact occur, wash affected area with plenty of water. **DO NOT PIPETTE REAGENTS BY MOUTH.**

Preparation Of Working Reagents

Catachem Total Protein Reagent is packaged ready to use. No preparation is required.

Reagent Storage And Stability

Store Catachem Total Protein reagents at room temperature (12-26°C). When stored as directed, the reagent is stable until the expiration date stated on the label. The Catachem Total Protein Reagents have been tested to reflect shipping conditions and are

stable for the lifespan of the product if frozen up to 5 times or upon reaching temperatures up to 40°C for up to one week.

Specimen Collection And Preparation

Test sera should be fresh, clear and non-hemolyzed. When blood is drawn, it should be processed as soon as possible and the serum should be isolated from the clot without delay. In separated, non-hemolyzed serum the total protein concentration is stable for a few days if stored at 2-8°C. To obtain longer stability, specimens should be frozen.

Interfering Substances

Various substances have been reported to interfere with the Total Protein Biuret Method. A summary of the influence of drugs on clinical laboratory procedures may be found by consulting D.S. Young, et al.(3)

Quality Control

To ensure optimal performance of these reagents and this procedure, we recommend systematic calibration using Catachem's Catacal (C1200-10). Assay performance should be monitored by running normal/abnormal controls concomitantly with samples. Catachem has optimized this assay using Catatrol Level I (C1200-11) and Catatrol Level II (C1200-12) and recommends their use for daily QC.

Expected Values

The normal range of this assay as performed below is 6.0 g/dL to 8.5 g/dL. These values serve as suggested reference points only. For veterinary samples, ranges will be dependent on the species under test. It is recommended that each laboratory establish the normal ranges for the species under study and for the geographic area in which the laboratory is located.

Directions For Use

Catachem Total Protein Working Reagent is packaged ready for use. No preparation is required. The Working Reagent is stable until the expiration date stated on the label when stored between 2 and 30°C and capped while not in use.

Procedure

Important: Read entire procedure instructions before proceeding with assay.

Materials Required (Not Provided)

Spectrophotometer	550 nm
Match cuvettes	1 cm light path
Timer	to time incubation time
Pipette	1.0 ml for reagent
Pipette	0.02 ml for sample

Materials Provided

Total Protein Reagent

Materials Required But Not Provided

Catachem Total Protein Calibrator (Catacal)
Catachem Total Protein Quality Control Standards: Catatrol I And Catatrol II



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Calibration

Catachem Calibrator (“Catacal”) is recommended for use in the Catachem Total Protein assay. The Calibrator and the unknown should be treated in the same way while performing the Total Protein procedure.

Analytical Parameters

Wavelength	550nm
Temperature	37°C
Pathlength	1 cm
Reaction Mode	Endpoint
Reaction Time	5.0 minutes
Reagent Volume	1.0 ml
Sample Volume	0.02ml
Total Volume	1.02ml
Sample to Rgt Ratio	1:51

Assay Procedures

1. Pipette 1.0ml of Catachem Total Protein Reagent into each of three cuvettes marked “Calibrator”, “Sample”, and “Blank”.
2. Pipette 0.020 ml of Calibrator or Sample into their respective cuvettes. Use 0.02 ml of de-ionized water for the blank. Mix all cuvettes well.
3. Incubate all cuvettes for 5.0 minutes at 37° C.
4. Set spectrophotometer wavelength at 550nm.
5. Read the “Calibrator”, “Sample”, and “Blank” absorbances.
6. Calculate the Total Protein concentration (g/dL) in the sample(s), as shown in calculations and results.

Calculations And Results

$$\text{Total Protein (g/dL)} = \frac{\text{Sample } \Delta \text{ OD}}{\text{Cal } \Delta \text{ OD}} \times \text{Calibrator (g/dL)}$$

Where: $\Delta \text{ OD} = \text{Assay OD} - \text{Blank OD}$
Cal = Calibrator
(eg Calibrator Value = 4.0 g/dL)

Example: Sample	$\frac{\Delta \text{ OD}}{0.25}$
Calibrator	0.20

$$\text{Total Protein (g/dL)} = \frac{0.25}{0.20} \times 4.0 \text{ g/dL}$$

$$= 5.0 \text{ g/dL}$$

NOTE: Samples with Total Protein concentrations greater than 10 g/dL should be diluted with physiological saline and reassayed. Results should be adjusted for dilution.

Method Performance Characteristics

Sensitivity: 0.0336 - 0.0410 absorbance units per g/dL.

Linear Range: 0-10 g/dL.

Precision: Within-run and day-to-day precision is summarized below.

Precision Study

T. PROT Mean g/dl	Within-Run Precision		Total Precision	
	SD g/dl	CV %	SD g/dl	CV %
2.8	0.10	3.4	0.10	3.50
5.9	0.11	2.1	0.12	2.10
8.7	0.11	2.0	0.17	2.00

Correlation

A comparison of this method using an automated clinical instrument was carried out against a reference biuret method resulted in the following regression statistics:

Range	=	3.9-10.0 g/dL
N	=	198
Y	=	0.92x + 0.48
r	=	0.982
Sy.x	=	0.19

References

1. Tietz, NW: Fundamentals of Clinical Chemistry. WB Saunders Co. (1976).
2. Weichselbaum TE: American Journal Clinical Pathology 16:40 (1946).
3. Young DS, Pestaner LC, Gibberman V: Effect of drugs on clinical laboratory tests. Clinical Chemistry 21 (5):1D-432D (1975).