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=0) 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 reactions that occur in this method:

Protein (-C=0; =N-N) + Biuret (Cu++)----->Chelate Complex

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

Biuret Reagent
One liter contains:

Cupric Sulfate .5H20	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.

Specimen Collection And Preparation
Test sera should be fresh, clear and unhemolyzed. 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, unhemolyzed 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 monitor the performance of the Working Reagents and the procedure used, we recommend the regular use of a normal and abnormal controls;

Expected Values
The range of expected values in humans determined for this method is 6.0 g/dL to 8.5 g/dL. These values are suggested guidelines. It is recommended that each laboratory establish the normal range for the species under test as well as for the 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, stored at 25˚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

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 37º 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: Δ OD = Assay OD -Blank OD
Cal = Calibrator
(eg Calibrator Value = 4.0 g/dL)

	Δ OD
Example: Sample	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	Within-Run Precision		Total Precision	
Mean	SD	CV	SD	CV
g/dl	g/dl	%	g/dl	%
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).

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