

Total Protein Reagent

Catalog #: 43722

for use with the

SDI CA480 Clinical Chemistry System

INTENDED USE

For the *in vitro* quantitative determination of total protein concentration in serum.

SUMMARY AND EXPLANATION ¹

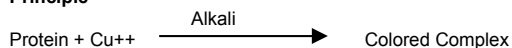
Total Protein is useful for monitoring changes in protein levels caused by various disease states. Hyperproteinemia is noted in dehydration, Addison's disease, or diabetic acidosis.

Hemodilution (increase in plasma water volume) is reflected as relative hypoproteinemia which occurs with water intoxication or salt retention syndromes, during massive intravenous infusions, and physiologically when recumbant position is assumed.

METHODOLOGY

The color reaction of protein molecules with cupric ions, known as the Biuret color reaction, has been known since 1878. Since the Riegler² publications of 1914, several attempts have been made to stabilize the cupric ions in the alkaline reagent. Kingsley,^{3,4} modified the procedure in 1939 and 1942 to include the use of sodium potassium tartrate as a complexing agent. This procedure was later modified by Weichselbaum⁵ and Gornall⁶. The present method is based on these modifications.

Principle



Protein in serum forms a blue colored complex when reacted with cupric ions in an alkaline solution. The intensity of the violet color is proportional to the amount of protein present when compared to a solution with known protein concentration.

REAGENT COMPOSITION

Active Ingredients	Concentrations
Sodium Hydroxide	600mM
Cupric Sulfate	12mM
Potassium Sodium Tartrate	32mM Potassium
Potassium Iodide	30mM

Precautions

1. This reagent is for *in vitro* diagnostic use only.
2. Avoid ingestion. DO NOT PIPETTE BY MOUTH. In case of ingestion drink large amounts of water and seek medical attention quickly.
3. Avoid contact with skin and eyes. The reagent contains sodium hydroxide which is corrosive. In case of contact with skin, flush with water. For eyes, seek medical attention.

REAGENT PREPARATION

Reagent is supplied ready to use.

REAGENT STORAGE

Store reagent at room temperature.

REAGENT DETERIORATION

The reagent should be a clear pale blue solution. Turbidity or the presence of a black precipitate indicates reagent deterioration and should not be used.

SPECIMEN COLLECTION AND STORAGE

1. Unhemolyzed serum is the specimen of choice.
2. Gross hemolysis will cause elevated results because of the released hemoglobin as well as the increase in background color.
3. Lipemic sera cause elevated results and should be run with a serum blank.
4. Samples with bromosulphthalein (BSP) will result in falsely elevated results.⁹
5. Protein in serum is stable for one week at room temperature (18 - 25°C) and for at least one month refrigerated (2 - 8°C) when guarded against evaporation.⁷

INTERFERENCES

Young,⁸ et al has reviewed a number of drugs and substances that may affect protein concentrations.

ADDITIONAL EQUIPMENT REQUIRED BUT NOT PROVIDED:

1. SDI CA480 Clinical Chemistry System
2. Iron-free deionized water and related equipment, e.g.: pipettes
3. Analyzer specific consumables, e.g.: sample cups
4. Control, and Calibrator materials such as those provided by SDI Biomed.

ASSAY PROCEDURE

	System Parameters
Total Protein	
TEMPERATURE:	37°C
WAVELENGTH:	540 nm
DIRECTION:	Increase
SAMPLE / RGT RATIO:	1 : 50
e.g. Sample Vol.	0.02 mL (20mL)
Reagent Vol.	1.0 mL
REACTION TIME:	5 Min

Procedure Notes

1. Final color is stable for 60 minutes at room temperature.
2. Serums with values above 15.0 g/dL should be diluted 1:1 with 0.9% saline, and the final answer multiplied by two.
3. The reagent and sample volumes may be altered proportionally to accommodate various instrument requirements.

Calculation

Abs = Absorbance

$$\frac{\text{Abs. of unknown}}{\text{Abs. of standard}} \times \text{conc. of std.} = \text{total protein (g/dL)}$$

Abs. of standard

Example:

$$\text{Absorbance of unknown} = 0.350$$

$$\text{Absorbance of standard} = 0.400$$

$$\text{Concentration of standard} = 8 \text{ g/dL}$$

Then:

$$0.350 \times 8 = 7.00 \text{ g/dL}$$

0.400

Limitations

1. Samples with values above 15.0 g/dL should be diluted 1:1 with 0.9% saline, re-run and result multiplied by two.
2. The Biuret procedure is not sensitive at low ranges (<1g/dL). Do not use for urine or spinal fluid.

CALIBRATION

Use an aqueous Total protein standard or an appropriate serum calibrator.

QUALITY CONTROL

The integrity of the reaction should be monitored by use of a two level control with known Total Protein values.

EXPECTED VALUES⁹

6.2 – 8.5 g/dL

1. The effect of posture, when blood is drawn, varies with the individual but recumbent values are usually lower than ambulatory. Differences may be as much as 1.2 g/dL.
2. It is strongly recommended that each laboratory establish its own normal range.

PERFORMANCE

Linearity:

When run as recommended the assay is linear to 1.0 – 15.0 g/dL.

Method Comparison:

Studies performed between this procedure and another procedure based on the same principle yielded the following results:

Correlation Coefficient:	0.9901
Slope:	1.011
Intercept:	0.19 (g/dL)

Precision:

Within Run	Level 1	Level 2
n=40		
Mean (g/dL)	6.24	8.11
S.D. (g/dL)	0.06	0.11
C.V. (%)	0.9	1.4

Total

n=40		
Mean (g/dL)	6.24	8.11
S.D. (g/dL)	0.10	0.13
C.V. (%)	1.6	1.6

REFERENCES

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Manufactured for:



23679 Calabasas Road, Unit 241
Calabasas, CA 91302
800-952-2470

PI: 43722.01

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