Pointe Total Protein (Biuret) Reagent Set

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Intended Use
For the quantitative determination of total protein concentration in serum using the Yumizen C230 and Yumizen C240 analyzers. **Rx Only.**

Method History
The color reaction of protein molecules with cupric ions, known as the Biuret color reaction, has been known since 1878. Since the Riegler[1] publications of 1914, several attempts have been made to stabilize the cupric ions in the alkaline reagent. Kingsley,[2,3] 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 Weichselbaum[4] and Gornall.[5] The present method is based on these modifications.

Principle
Alkali

Protein + Cu^{++} ----------➢ Colored Complex

Protein in serum forms a violet 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 Content
Sodium Hydroxide 600mM, Copper Sulfate 12mM, Sodium Potassium Tartrate 32mM, Potassium Iodide 30mM, Non-reactive ingredients.

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 comes in a ready to use form.

Reagent Storage
Store reagent at room temperature(15-30˚C). The reagent is stable until the expiration date appearing on the label when stored as directed.

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. A serum Blank should be performed.
4. Samples with bromosulfophthalein (BSP) will result in falsely elevated results.[8]
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.[6]

Interferences
Young, et al.[7] has reviewed a number of drugs and substances that may affect protein concentrations.

Materials Provided
Total Protein reagent

Materials Required but not Provided
1. Yumizen C230 / Yumizen C240 Analyzer
2. Yumizen C230 / Yumizen C240 Operation Manual
3. Pointe Chemistry Calibrator, catalog number C7506-50
4. Pointe Chemistry control, catalog number C7592-100

Test Parameters

Test:	TP	Chemistry:	Total Protein
Chemistry No.:	229	Print Name:	Total Protein
Reaction Type:	Endpoint	Reaction Direction:	Positive
Pri. Wave:	546 nm	Sec. Wave:	670 nm
Decimal.:	0.1	Samp. Type:	Serum
Blank Time:		Reaction Time:	10 11
Unit:	g/dL	Incubation Time:	0

	Sample Vol.	Aspirated	Diluent		Reagent Vol.	Diluent	
Standard;	4	uL	uL	uL	180	uL	uL
Decreased;		uL	uL	uL			
Increased;		uL	uL	uL			

Linearity Range (Standard);	1.0-15.0		Linearity Limit:		
Linearity Range (Decreased):			Substrate Depletion:		
Linearity Range (Increased):			Mixed Blank Abs.:	-40000	40000
R1 Blank Abs.:	-40000	40000	On-board Stability:	30	Day (s)
Blank Response	-40000	40000	Reagent Alarm Limit:	5	
Twin Chemistry:					

Prozone Check:		
Q1:	Q2:	Q3:
Q4:	PC:	ABS:

Use Qualitative Result:		
	Range:	Flag:

Slope Offset:			
	Slope	Offset	Unit
	1	0	g/dL

Pretreatment:			
Pretreat Sample Vol.:	uL	Pretreat Reagent Vol.:	uL

Ref. Range:					
Sample Type:	Gender:	Age Range:	Ref. Range:	Critical Range:	Unit:

Pointe
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Calibration Setup Parameters

Chem: TP
Calibration Setting
Math Model: Two-Point Linear
Factor: Replicates: 2
Acceptance Limits
Cal Time: 336 hr.
Slope Diff: SD:
Sensitivity: Repeatability:
Deter Coeff:
Auto Calib.
☐ Cal Time

Calibrator	Conc.	Pos	Lot No.
Water	0.0	W	
Chem Cal	*	*	

* User Defined

Calibration

Use an NIST-traceable serum calibrator. The procedure should be calibrated according to the instrument manufacturer's calibration instructions. If control results are found to be out of range, the procedure should be re-calibrated.

Quality Control

1. Use control sera with known total protein concentrations to monitor the integrity of the reaction.
2. Quality control requirements should be performed in conformance with local, state, and/or Federal regulations or accreditation requirements.

Calculation (Example)

Abs. = Absorbance

$$\frac{\text{Abs. of Unknown}}{\text{Abs. of Standard}} \times \text{Conc. of standard} = \text{Total Protein (g/dl)}$$

Example: Abs. of Unknown = 0.350, Abs. of Standard = 0.400
Concentration of Standard = 8 g/dl

Then: $\frac{0.350}{0.400} \times 8 = 7.00$ g/dl

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 (<1 g/dl). Do not use for urine or spinal fluid.

Expected Values[8]

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 range.

Performance

1. Linearity: 1.0 – 15.0 g/dl
2. Comparison: A study was performed between the Yumizen 200 series analyzers and a similar analyzer and method, resulting in a correlation coefficient of 0.996 with a regression equation of y=0.919 x + 0.36.
3. Precision: Precision studies were performed using the Yumizen 200 series analyzers following a modification of the guidelines which are contained in NCCLS document EP5-T2.[9]

Within Run			Day to Day		
Mean	S.D.	C.V.%	Mean	S.D.	C.V.%
4.77	0.13	2.7	4.78	0.07	1.5
7.46	0.16	2.1	7.63	0.14	1.8

References

1. Riegler, E., Anal. Chem. 53:242 (1914).
2. Kingsley, G.R., J. Biol. Chem. 131:197 (1939).
3. Kingsley, G.R., J. Lab. Clin. Med. 27:840 (1942).
4. Weichselbaum, T., Amer. J. Clin. Path. 16:40 (1946).
5. Gornall, A., et al, J. Biol. Chem. 177:752 (1949).
6. Henry, R.J., et al, Clinical Chemistry: Principles and Technics, Harper & Row, New York, p.415 (1974).
7. Young, D.S., et al, Clin. Chem. 21:1D (1975).
8. Tietz, N.W., Fundamentals of Clinical Chemistry Philadelphia, W.B. Saunders, pp. 299, (1976).
9. NCCLS document "Evaluation of Precision Performance of Clinical Chemistry Devices", 2nd Ed. (1992).

Symbol Key

Use by (YYYY-MM-DD)	LOT Lot and batch code
REF Catalog number	Manufacturer
IVD *In vitro* diagnostic medical device	Temperature limitation
Consult instructions for use	**Rx Only:** Prescription Use Only
CE mark	EC REP Authorized representative in the European Community

REF 12-T7528-120

Manufactured by
HORIBA Instruments Incorporated - Pointe Brand
5449 Research Drive Canton, MI 48188

IVD

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Certified to Perform Reagents

The Pointe reagents are certified to be manufactured according to specified parameters. Any Pointe reagent product not meeting specifications through its listed expiration date will be remedied immediately without charge.

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