Total Protein (Biuret) Reagent Set

Intended Use
For the quantitative determination of total protein concentration in serum using the Mindray BS-480 analyzer.

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
$$\text{Protein} + Cu^{++} \xrightarrow{\text{Alkali}} \text{Colored Complex}$$

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 and Hazards
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.

Hazards:

Hazard Classifications: Skin Corrosion/Irritation (Category 1), Serious eye damage/eye irritation (Category 1)
Hazard Statements: H314: Causes severe skin burns and eye damage, H318 : Causes serious eye irritation
Precautionary Statements: **Prevention:** P260: Do not breathe dust/fume/gas/mist/vapors/spray. P264: Wash skin thoroughly after handling. P280: Wear protective gloves/protective clothing/eye protection/face protection. **Response:** P310: Immediately call a POISON CENTER or doctor/physician. P363: Wash contaminated clothing before reuse. P301+P330+P331 : If SWALLOWED : Rinse mouth. Do NOT induce vomiting. P303+P361+P353 : IF ON SKIN (or hair) : Remove/Take off immediately all contaminated clothing. Rinse SKIN with water/shower. P304+P340: IF INHALED: Remove victim to fresh air and keep at rest in a position comfortable for breathing. P305+P351+P338: IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. **Storage:** P404 : Store in a closed container. **Disposal:** P501 : Dispose of contents into sewer system after diluting with large volumes of water, if in accordance with local regulations. **Refer to the Safety Data Sheet for this product (SDS-TP600) available at www.medtestdx.com.**

Signal Word: Danger

Reagent Preparation
Reagent comes in a ready to use form.

Reagent Storage and Stability
Store reagent at room temperature (15-30˚C). The reagent is stable until the expiration date appearing on the label when stored as directed. Manufacturer studies have shown reagent is stable for 30 days once placed in the refrigerated reagent carousel (2-10°C).

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

Total Protein (Biuret)
Reagent Set

Materials Required but not Provided
1. Mindray BS-480 Analyzer
2. BS-480 operation manual
3. Chemistry Calibrator, catalog number CHEC480
4. Chemistry control, catalog number CHEQ480

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 test may need to be re-calibrated. Under typical operating conditions manufacturer calibration stability studies have shown the calibration curve will be stable for at least 14 days.

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.

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. Assay Range: 1.0 – 15.0 g/dL
2. Correlation: A study was performed between the Mindray BS-480 and a similar analyzer using this method, resulting in the following:

Method	Total Protein
N	84
Mean Total Protein (g/dL)	7.11
Range (g/dL)	3.7-9.6
Standard Deviation	1.36
Regression Analysis	y = 0.937x + 0.11
Correlation Coefficient	0.9969

3. Precision: Precision studies were performed using the Mindray BS-480 analyzer following a modification of the guidelines which are contained in NCCLS document EP5-T2.[9]

Within Day

Sample	LOW	MID	HIGH
N	20	20	20
Mean	3.40	6.99	11.51
Standard Deviation	0.00	0.03	0.03
Coefficient of Variation (%)	0.0%	0.4%	0.3%

Total

Sample	LOW	MID	HIGH
N	40	40	40
Mean	3.40	6.95	11.64
Standard Deviation	0.12	0.12	0.23
Coefficient of Variation (%)	3.5%	1.7%	2.0%

4. Sensitivity: 2SD limit of detection (95% Conf) = 0.0 g/dL

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).

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CHEMISTRY PARAMETERS

Chem:	TP	No.:	229	Sample Type:	Serum
Chemistry:	Total Protein			Print Name:	TP
Reaction Type:	End Point			Reaction Direction:	Positive
Pri Wave:	546			Sec Wave:	660
Unit:	g/dL			Decimal	0.1
Blank Time:	10 12			Reaction Time:	33 35

	Sample Vol.	Aspirated	Diluent	Reagent Vol.	Diluent
Standard:	2.7 ul	--- ul	--- ul	R1: 120 ul	--- ul
Decreased:	--- ul	--- ul	--- ul	R2: --- ul	-- ul
Increased:	--- ul	--- ul	--- ul	R3: --- ul	-- ul
	☐ Sample Blank	☑ Auto Rerun		R4: --- ul	--- ul

Slope/Offset Adjustment

Slope: 1 Offset: 0

Linearity Range (Standard)	1	15		Linearity Limit:
Linearity Range (Decreased)	____	____		Substrate Depletion:
Linearity Range (Increased)	____	____		Mixed Blank Abs:
R1 Blank Abs:	____	____		Uncapping Time
Blank Response:	____	____		Reagent Alarm Limit:
Twin Chemistry:				☐ Enzyme Linear Extension

☐ Prozone Check ○ Rate Check ○ Antigen Addition

Q1: Q2: Q3: Q4:

PC: ABS:

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CALIBRATION PARAMETERS

Calibrator Definition

Calibrator:	*	Lot No.:	*
Exp Date:	*		

Carousel	**Pos**
Sample Carousel 1	*
Sample Carousel 2	
Sample Carousel 3	

Reagent/Calibration

Calibrator	Pos	Lot No	Exp Date	Chem	Conc	Unit
Water	W	*	*	TP	0	g/dL
Chemistry Calibrator	*	*	*	TP	*	g/dL

Calibration Setup

Chem: TP

Calibration Settings

Math Model: Two-Point Linear

Factor: Replicates: 2

Acceptance Limits

Cal Time:	*	Hour	
Slope Diff:	---	SD:	---
Sensitivity :	---	Repeatability:	---
Deter Coeff:	---		

Auto Calib.

☐ Bottle Changed ☐ Lot Changed ☐ Cal Time

It is recommended that two levels of control material be assayed daily.
* Indicates user defined parameter.

REF TP480

Manufactured for MedTest DX
5449 Research Drive Canton, MI 48188

IVD

Symbol Key

Use by (YYYY-MM-DD)	LOT Lot and batch code	REF Catalog number	Manufacturer
Temperature limitation	Consult instructions for use	IVD *In vitro* diagnostic medical device	