



# VDx TOTAL PROTEIN

## (BIURET Method)

Liquid Reagent

### INTENDED USE:

This reagent kit is used for *in-vitro* quantitative determination of Total Protein in serum and plasma.

### TEST PRINCIPLE:

In alkaline medium, peptide bonds of proteins react with cupric ions in Biuret reagent to form violet coloured complex with an absorption maximum at 546 nm (530 - 570 nm). Intensity of the colour formed is directly proportional to the concentration of total proteins in the sample.

### KIT CONTENTS:

**REAGENT 1:** Biuret Reagent

**REAGENT 2:** Calibrator (Conc. as indicated on the label)

Insert : 01 No.

### PREPARATION OF THE WORKING REAGENT:

Reconstitute reagents as per instructions on individual bottle labels of reagent 1 to prepare working reagent.

### STORAGE AND STABILITY:

VDx Total Protein Liquid reagents are stable till the expiry date indicated on the labels when stored at 2-8°C. The working reagent is stable for six months at room temperature. Use distilled water of good quality for reconstitution to avoid precipitation of the reagent.

### SPECIMEN COLLECTION & STORAGE :

Serum or heparinised plasma can be used. Samples should preferably be used on the same day. If necessary, may be preserved upto one week if stored at 2-8°C. Sample should be brought to room temperature prior to use.

### PRECAUTIONS:

1. For *in-vitro* diagnostics use only.
2. Do not inhale the reagent or pipette by mouth.
3. Do not freeze or expose the reagents to higher temperature as it may affect the performance of the kit.
4. Before the assay bring all the reagents to room temperature.
5. Avoid contamination of the reagent during assay process.
6. Use clean glassware free from dust or debris.
7. Do not use the reagent if the reagent is hazy or cloudy.

### PROCEDURE (Automated):

Refer to specific instrument application instructions.

### TEST PROCEDURE (Manual):

Pipette into Test Tube	Blank	Standard	Test
Biuret reagent	1 ml	1 ml	1 ml
Calibrator	-	20 µl	-
Sample	-	-	20 µl
Distilled water	20 µl	-	-

Mix and allow to stand at room temperature (21-25°C) for 20 minutes or 5 minutes at 37°C. Read absorbance of the Test ( $A_T$ ), Calibrator ( $A_C$ ) and Blank ( $A_B$ ) at 546 nm (530 to 570 nm) or with Green filter against distilled water.

### CALCULATIONS:

$$\text{Total Proteins (gm/dl)} = \frac{A_T - A_B}{A_C - A_B} \times \text{Total Protein conc. provided on calibrator label}$$

### EXPECTED VALUES\*:

Total Proteins : 6.0 - 8.5 gm/dl

\*It is recommended that each laboratory should establish its own normal range representing its patient population.

### PERFORMANCE:

1. **Linearity:** 10 gm/dl

2. **Comparison:**  $r = 0.98$

$$y = 1.4x + 0.6$$

3. **Precision:**

	Within Run			Run to Run		
	Mean	S.D.	C.V.%	Mean	S.D.	C.V.%
Low	4.1	0.6	1.1	4.6	0.6	2.1
High	7.1	0.2	1.1	7.6	0.4	2.2

4. **Specificity:**

VDx Total Protein Liquid reagent is free of interference from moderate levels of hemoglobin, bilirubin, lipids and salicylates. Avoid use of grossly hemolysed samples or lipemic sera.

### CLINICAL SIGNIFICANCE:

Elevated levels of total proteins are associated with dehydration due to vomiting and diarrhoea, multiple myeloma, chronic liver diseases and chronic infections. Decreased levels are found in renal diseases, malnutrition, albuminuria and terminal liver failure. Increase in total protein levels is generally due to an increase in total globulin with the concentration of albumin remaining normal or decreasing marginally.

### AUTOMATED APPLICATIONS:

VDx Total Protein Liquid reagents can be used with Hitachi 700 series, RA 1000, 2000, XT, Express 550 plus, Synchron CX4, Lisa 200, BTR 810/820/830, RA50, Erbachem-5 plus etc. Application sheets for use on specific semi automatic, batch and auto analyzers are available on request. Input parameters for semi auto/auto analysers are given below :

INPUT PARAMETERS	VALUES
Type of reaction	End point
Wavelength	546 nm.
Incubation time	20 minutes/5 minutes
Calibrator concentration	Provided on the Label
Temperature	21-25°C/37°C
Units	gm/dl
Upper Normal value	8.5 gm/dl
Lower Normal value	6.0 gm/dl

INPUT PARAMETERS	VALUES
Linearity	10 gm/dl
Reagent volume	1. ml
Calibrator/Sample volume	20 $\mu$ l

**QUALITY CONTROL:**

For accuracy, it is necessary to run known serum controls with each assay.

**REFERENCES :**

- Vatzidis, H. (1977), Clin. Chem. 23:908
- Weichselbaum, T.E. (1957) Am. J. Clin. Path. 16:40-48.