MEDICA

℞Only IVD CE

REF 10213-4 4 x 20 mL / 5 mL

TOTAL PROTEIN (TP)

Each wedge contains a usable volume of 20 mL of R1 reagent and 5 mL of R2 reagent.

INTENDED USE

The EasyRA TP reagent is intended for the quantitative measurement of total protein in human serum or plasma, using the MEDICA EasyRA® clinical chemistry analyzer.
For *in vitro* diagnostic use. For professional use only.

SUMMARY AND EXPLANATION[1,2,3]

In human plasma, serum protein is comprised mainly of albumin, which makes up 50 to 60% of total protein, with the remainder comprised of α1-, α2-, β- and γ-globulins. The concentration of total protein (TP) is important in maintaining the normal balance and the exchange of water between the blood and tissues. A low serum protein concentration may be due to malabsorption, impaired synthesis, or protein loss due to hemorrhages or excessive catabolism, as seen in renal and hepatic disorders, which may result in hypoproteinemia. In the case of hyper-immunoglobulinemia (e.g. multiple myeloma and infection) or dehydration, hyperproteinemia may occur.

PRINCIPLE OF THE PROCEDURE

The assay method of measuring serum protein is by means of a biuret reaction, whereby cupric ions in an alkaline solution react with compounds (containing two or more amide or peptide bonds linked to a carbon atom), to form a colored complex.[4]

$$\text{Serum Proteins} + Cu^{++} \xrightarrow{\text{Alkaline pH}} \text{Violet-colored complex}$$

The violet-colored complex is measured spectrophotometrically at 550 nm with 700 nm as a blanking wavelength, The intensity of the complex is directly proportional to the protein concentration in the sample.[5]

REAGENTS

TP Reagent (R1):

Sodium Hydroxide	0.4 g/dL
Potassium sodium tartrate	17 mmol/L

TP Reagent (R2):

Sodium Hydroxide	500 mmol/L
Potassium sodium tartrate	80 mmol/L
Potassium iodide	75 mmol/L
Copper sulphate	0.5 g/dL

PRECAUTIONS

1. Good laboratory safety practices should be followed when handling any laboratory reagent. (CLSI, GP17-A3).
2. Sodium Hydroxide is corrosive and causes burns. Do not pipette reagents by mouth. If swallowed, seek medical advice immediately. Avoid eye and skin contact. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. If skin contact occurs, wash skin for at least 15 minutes.
3. As with any diagnostic test procedure, results should be interpreted considering all other test results and the clinical status of the patient.
4. Do not use washed cuvettes.

INSTRUCTIONS FOR REAGENT HANDLING, STORAGE AND STABILITY

The reagent is ready to use as supplied. Unopened reagent is stable until the expiration date listed on the label if stored at 4–25ºC. The reagent is stable on-board the EasyRA clinical chemistry analyzer for the number of days programmed on the RFID chip on the reagent wedge if the reagent is removed at the end of the day and stored overnight at 18–25ºC. Do not use the reagent if it is turbid or cloudy or if it fails to recover known serum control values. Protect from light.

SPECIMEN COLLECTION AND STORAGE / STABILITY[2, 6]

Clear non-hemolyzed serum or plasma should be used. Lithium heparin coated tubes may be used for plasma collection. Total Protein in serum or plasma is stable for 7 days at 2 – 8°C and 2 months at -20°C.[7]

PROCEDURE

Materials Provided
Medica TP Reagent Wedge, REF 10213-4

Additional Materials Required
Medica EasyCal® Chemistry, REF 10651
Medica EasyQC® Chemistry/Electrolytes – Level A, REF 10793
Medica EasyQC® Chemistry/Electrolytes – Level B, REF 10794
Medica Precision Test Dye Wedge, REF 10764
Medica Cleaner Wedge – Chemistry & ISE, REF 10660 *or*
Medica Cleaner Wedge – Chemistry, REF 10661

Instructions for Use
The reagent is ready to use as supplied. Remove the cap and place the reagent in the EasyRA clinical chemistry analyzer reagent tray located in the reagent area. Opened reagent is stable on-board the EasyRA clinical chemistry analyzer, for the number of days programmed on the RFID chip on the reagent wedge (60 days maximum) when removed at the end of the day and stored at 18 - 25ºC (capped) after first opened.

Note: Check inside the neck of the wedge for foam after removing the cap and placing the wedge on the analyzer. If there is foam, remove it with a swab or a disposable pipette before performing the test.

Calibration
Medica EasyCal Chemistry (REF 10651) is recommended for the calibration of the assay. The calibration interval (60 days maximum) is programmed on the RFID chip on the reagent wedge. Recalibration is required whenever there is a change in reagent lot number or if a shift in quality control values occurs.

Quality Control
It is recommended that two levels of human serum-based controls (normal and abnormal) be run with the assay daily whenever patient testing is performed and with each reagent lot change. Failure to obtain the proper range of values in the assay of control material may indicate reagent deterioration, instrument malfunction, or procedural errors. The laboratory should follow local, state and federal quality control guidelines when using quality control materials.

Results
After completion of the assay, the EasyRA clinical chemistry analyzer calculates the total protein (TP) concentration from the ratio of the corrected absorbance of the unknown sample to the corrected absorbance of the calibrator, multiplied by the calibrator value.

$$\text{TP (g/dL)} = \frac{[(A_U - A_{Blk})_{550} - (A_U - A_{Blk})_{700}]}{[(A_C - A_{Blk})_{550} - (A_C - A_{Blk})_{700}]} \times \text{Cal Value}$$

Where A_U and A_C are the absorbance values of unknown and calibrator, respectively; A_{Blk} is the absorbance of the reagent blank; and "Cal Value" is the concentration of Total Protein (TP) in the calibrator (g/dL).

EXPECTED VALUES[8]
The reference range for TP in serum is as follows:

Normal: 6.3 – 8.3 g/dL

These values are guidelines. It is recommended that each laboratory establish its own range of normal values since differences exist among instruments, laboratories and local populations.

Procedural Limitations (e.g. if sample is above assay range)
The EasyRA clinical chemistry analyzer flags any result above 10 g/dL as Linearity High "LH." If the "Re-run" icon is selected by the operator, the sample may be re-tested using one half (1/2) the sample volume. The retest results are calculated to reflect the use of the smaller sample volume. This will extend the reportable range of the TP test to 20 g/dL.

PERFORMANCE CHARACTERISTICS[9]

Reportable Range
The reportable range is 0.1 to 10 g/dL. Extended range is 0.1 to 20 g/dL when half of the sample is used (1:1 dilution).

Inaccuracy / Correlation (CLSI, EP09-A2)
The following table lists the data obtained in a comparison of the Medica reagent for TP (y) on the EasyRA clinical chemistry analyzer to the performance of a similar TP reagent (x) on the Roche COBAS MIRA* analyzer. The data shown below represents single determinations obtained on the EasyRA clinical chemistry analyzer vs. the average of two replicate values obtained on the COBAS MIRA analyzer.

Number of Samples	46	Range of Samples	0.5 to 8.4 g/dL
Slope	1.04	y Intercept	-0.02
Correlation Coefficient	0.9925	Regression Equation	Y = 1.04*X – 0.02

The following table lists the data obtained in a comparison of matched serum (x) and the lithium heparinized plasma (y) samples using the Medica reagent for Total Protein on the EasyRA clinical chemistry analyzer. The data below represents a single plasma determination vs. the average of two replicate serum values.

Number of Samples	62	Range of Samples	0.5 to 8.6 g/dL
Slope	1.0088	y Intercept	0.0265
Correlation Coefficient	0.9883	Regression Equation	Y = 1.0088*X + 0.0265

**Cobas Mira is a registered trademark of Roche Diagnostics, INC., Indianapolis, IN.*

Imprecision (CLSI, EP05-A2)
Within run imprecision: Five replicates of each of three levels of commercial human serum-based QC material were tested per day over 5 days.

QC Level g/dL	Within Run SD g/dL	Within Run CV %
5.9	0.05	0.8
4.6	0.05	1.1
4.3	0.05	1.2

Total Imprecision**:** Duplicate measurements of each of three levels of QC material were tested twice a day for 20 days.

QC Level g/dL	Total Imprecision SD g/dL	Total Imprecision CV %
6.0	0.09	1.4
4.7	0.07	1.4
4.1	0.18	4.3

Linearity (CLSI, EP06)

Linear from 0.1 to 10 g/dL, based on the linear regression Y = 1.004*X + 0.025.

Interfering Substances (CLSI, EP07)
Less than 10% interference was classified as “no significant interference.”
No significant interference was found up to 300 mg/dL hemoglobin. Do not use hemolyzed samples.
No significant interference was found up to 20 mg/dL total bilirubin.
No significant interference was found up to 300 mg/dL triglyceride (using Intralipid*). Do not use lipemic samples. If lipemic samples must be analyzed, use a lipid-clearing agent in combination with a centrifuge to clear the triglycerides in the sample.
No significant interference was found up to 30 mg/dL ascorbic acid.

**Intralipid is a registered trademark of Pharmacia AB, Clayton, NC*

Young provides a list of drugs and other substances that interfere with clinical chemistry tests.[10, 11]

REFERENCES

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2. Tietz NW. Editor, *Clinical Guide to Laboratory Tests*, 3rd ed. WB Saunders and Co., Philadelphia, PA, (1995), p. 518.
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7. Gunder WG, Zawta B et el. *The Quality of Diagnostic Samples*. 3rd ed: 2010: p 58-9.
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9. Data on file at Medica.
10. Young DS. *Effects of Drugs on Clinical Laboratory Tests* 4th ed. Washington, DC: AACC Press; 1995.
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EASYRA ASSAY PARAMETERS (TP)

Primary wavelength (nm)	550
Secondary wavelength (nm)	700
Reaction type	Endpoint (2)
Reaction direction	Increase
Reagent blank	Yes (with each calibration)
Sample blank	No
Reaction time	5.2 min
Calibration interval (maximum)	60 days
Reagent on-board stability	60 days

Serum/Plasma

Sample volume (µl)	3.0
Diluent volume (µl)	20
Reagent volume R1 (µl)	120
Reagent volume R2 (µl)	30
Decimal places (default)	1
Units (default values)	g/dL
Dilution factor	1:1 (to extend measuring range)
Linearity	0.1 to 10 g/dL