TOTAL PROTEIN

OSR6132	**4 x 25 mL**	**R1**
	4 x 25 mL	**R2**
OSR6232	**4 x 48 mL**	**R1**
	4 x 48 mL	**R2**
OSR6632*	**4 x 164 mL**	**R1**
	4 x 164 mL	**R2**

Intended Use
System reagent for the quantitative determination of Total Protein in human serum on Beckman Coulter AU analyzers.
*Total Protein reagent OSR6632 for use on the AU2700/5400 system only.

Summary
Measurements of Total Protein are used in the diagnosis and treatment of a variety of diseases involving the liver, kidney, or bone marrow as well as other metabolic and nutritional disorders.

The total serum protein is the sum of all circulating proteins and is a major component of blood. It is often useful, however, in interpreting the significance of the total protein concentration to have more specific knowledge of individual fractions such as albumins and globulins.[1]

Methodology
This Total Protein procedure is based on the modification of Weichselbaum.[2] Cupric ions in an alkaline solution react with proteins and polypeptides containing at least two peptide bonds to produce a violet colored complex. The absorbance of the complex at 540/660 nm is directly proportional to the concentration of protein in the sample.

$$\text{Protein} + Cu^{2+} \xrightarrow{OH^-} \text{Blue violet complex}$$

System Information
For AU400/400^e/480, AU600/640/640^e/680 and AU2700/5400 Beckman Coulter Analyzers.

Reagents
Final concentration of reactive ingredients:

Sodium hydroxide	200 mmol/L
Potassium sodium tartrate	32 mmol/L
Copper sulfate	18.8 mmol/L
Potassium iodide	30 mmol/L

Precautions
1. For *in vitro* diagnostic use.
2. WARNING! CORROSIVE! Do not pipet by mouth. Avoid contact with eyes, skin or clothing. In case of contact, immediately flush affected areas with plenty of water for 15 minutes. Obtain medical attention immediately for eye contact or ingestion.

Preparation of Reagents
For OSR6132 and OSR6232, the Total Protein Reagents are ready for use. No preparation is required. For OSR6632, insert the pipe supplied into the 180 mL reagent vial before use on the analyzer. Care must be taken when handling the pipe to avoid contamination. The pipe is for single use only. Do not remove the large cap.

Storage and Stability
1. The unopened reagent is stable until the expiration date printed on the label when stored at 2 - 25°C.
2. Opened reagents are stable for 30 days when stored in the refrigerated compartment of the analyzer.

Indications of Deterioration
Visible signs of microbial growth, turbidity, precipitate, or any change in color in the Total Protein reagent may indicate degradation and warrant discontinuance of use. R1 should be a clear blue solution and R2 should be a clear, colorless solution.

Specimen Collection and Preparation
Serum samples, free from hemolysis, are the recommended specimens. Plasma is not recommended since fibrinogen in the sample will add to the protein measured. If plasma must be used, the recommended anticoagulant is heparin.

Sample Storage and Stability
Total Protein is stable in serum for one week at room temperature (15 - 25°C) and for one month refrigerated (2 - 8°C).[3]

Interfering Substances
Results of studies[4] show that the following substances interfere with this Total Protein procedure.

The criteria for no significant interference is recovery within 10% of the initial value.

Bilirubin:	No significant interference up to 40 mg/dL Bilirubin
Hemolysis:	No significant interference up to 500 mg/dL Hemolysate
Lipemia:	No significant interference up to 1000 mg/dL Intralipid*

Total Protein

* Intralipid, manufactured by KabiVitrium Inc., is a 20% IV fat emulsion used to emulate extremely turbid samples.

The information presented is based on results from Beckman Coulter studies and is current at the date of publication. Beckman Coulter Inc. makes no representation about the completeness or accuracy of results generated by future studies. For further information on interfering substances, refer to Young[5] for a compilation of reported interferences with this test.

Procedure

A complete list of test parameters and operational procedure can be found in the User's Guide appropriate to the analyzer.

Materials Provided
Total Protein Reagent
Pipe (one per each 180 mL vial)

Materials Required But Not Provided
Calibrator (Cat. # DR0070)

Stability of Final Reaction Mixture
The Beckman Coulter AU analyzer automatically computes every determination at the same time interval.

Calibration
Calibration of this total protein procedure is accomplished by the use of the Chemistry Calibrator (Cat # DR0070), which is traceable to the National Institutes of Standards and Technology (NIST) Standard Reference Material (SRM) 927a.

Recalibration of this test is required when any of these conditions exist:
1. An observed drift in QC values of > 5%.
2. A change of bottle/Lot number.
3. Major preventative maintenance was performed on the analyzer.
4. A critical part was replaced.

Quality Control
During operation of the Beckman Coulter AU analyzer, at least two levels of an appropriate quality control material should be tested a minimum of once a day. In addition, controls should be performed after calibration, with each new lot of reagent, and after specific maintenance or troubleshooting steps described in the appropriate User's Guide. Quality control testing should be performed in accordance with regulatory requirements and each laboratory's standard procedure.

Results
Automatically printed out for each sample in g/dL at 37°C. For SI units (g/L) results must be multiplied by 10.

Dynamic Range

The Total Protein procedure is linear from 3 to 12 g/dL for serum determinations. Samples exceeding the upper limit of linearity should be diluted and repeated. The sample may be diluted, repeated and multiplied by the dilution factor automatically utilizing the AUTO REPEAT RUN.

Expected Values

3 years to adult:[6]	6.0 - 8.3 g/dL
Newborns:[6]	4.6 - 7.0 g/dL
Beckman Coulter Determined Reference Range:[7]	6.4 - 8.9 g/dL

Expected values may vary with age, sex, diet and geographical location. Each laboratory should determine its own expected values as dictated by good laboratory practice.

Specific Performance Characteristics

The following data was obtained using the Total Protein Reagent on Beckman Coulter AU analyzers according to established procedures. Results obtained in individual laboratories may differ.

Precision[9]
Estimates of precision, based on CLSI recommendations[8], are consistent with typical performance. The within run precision is less than 3% CV and the total precision is less than 4% CV. Assays of control sera were performed and this data reduced following CLSI guidelines above.

N = 80	Within run		Total	
Mean, g/dL	SD	CV%	SD	CV%
3.6	0.02	0.50	0.03	0.84
7.3	0.03	0.34	0.05	0.70
11.0	0.03	0.26	0.07	0.64

Method Comparison[9]
Patient samples were used to compare this Total Protein Reagent. The table below demonstrates representative performance on AU analyzers.

Y Method	AU640
X Method	Method 2
Slope	0.976
Intercept	+ 0.09
Correlation Coeff. (r)	0.9996
No. of Samples (N)	121
Range (g/dL)	3.1-11.7

Sensitivity
Typical change in absorbance per minute for 1 g/dL of Total Protein is 23.7 mAbsorbance in the AU400/400[e], 51.3 mAbsorbance in the AU600/640/640[e], and 62.5 mAbsorbance in the AU2700/5400/680/480 analyzers.

References

1. Bakerman, S., A,B,C's of Interpretive Laboratory Data, 2nd Edition, Griffin & Tilghman, 347, 1984.
2. Weichselbaum, T.E., Amer J Clin Path, 16: 40, 1946.
3. Kaplan, L.A. and Pesce, A.J. (eds), Clinical Chemistry Theory, Analysis and Correlation, 3rd Edition, C.V. Mosby Co., 1996.
4. CLSI/NCCLS, Interference Testing in Clinical Chemistry EP07-A, 2002.
5. Young, D. S., Effects of Drugs on Clinical Laboratory Tests, 5th Edition, 2000.
6. Tietz, N. W. (ed), Clinical Guide to Laboratory Tests, 3rd Edition, WB Saunders, 1990.
7. Beckman Coulter Inc. data on samples collected from 200 blood donors in North Texas.
8. CLSI/NCCLS Evaluation Protocol EP5-A, 1999.
9. Data is on file for specific AU analyzers.

Manufactured by: Beckman Coulter, Inc., 250 S. Kraemer Blvd. Brea, CA 92821, USA

CE