Intended Use
System reagent for the quantitative determination of Ceruloplasmin (CER) in human serum on Beckman Coulter AU analyzers.

Summary
Ceruloplasmin is the primary copper containing protein in plasma. It is a late acute phase reactant synthesized by the liver. Acute phase reactant refers to proteins whose serum concentrations rise significantly during acute inflammation due to causes including surgery, myocardial infarction, infections and tumors. Ceruloplasmin’s main clinical importance is in the diagnosis of Wilson’s disease. Here plasma Ceruloplasmin concentration is reduced while dialyzable copper concentration is increased. Increased ceruloplasmin levels are particularly notable in diseases of the reticuloendothelial system such as Hodgkin’s disease as well as during pregnancy or the use of contraceptive pills. Low plasma levels of ceruloplasmin are found in malnutrition, malabsorption, nephrosis and severe liver disease, particularly biliary cirrhosis.1,2

Methodology
Immune complexes formed in solution scatter light in proportion to their size, shape and concentration. Turbidimeters measure the reduction of incident light due to reflection, absorption, or scatter.

In the procedure, the measurement of the decrease in light transmitted (increase in absorbance) through particles suspended in solution as a result of complexes formed during the antigen-antibody reaction, is the basis of this assay.

System Information
For AU400/400e/480, AU680/640/640e/680 and AU2700/5400 Beckman Coulter Analyzers.

Reagents
Final concentration of reactive ingredients:
Solution of Polymers in Phosphate Buffered Saline (pH 7.4 – 7.6)
Rabbit anti-human Ceruloplasmin antiserum
Also contains preservatives.

Precautions
1. For in vitro diagnostic use.
2. Do not ingest. Harmful if swallowed.
3. Contains sodium azide as a preservative which may react with lead joints in copper plumbing to form explosive compounds. Even though the reagent contains minute quantities of sodium azide, drains should be well flushed with water when discarding the reagent.

Preparation of reagents
The reagents are ready to use. No preparation is required.

Storage and stability
1. The unopened reagents are stable until the expiration date printed on the label when stored at 2 - 8°C.
2. Opened bottles of reagent are stable for 90 days when stored in the refrigerated compartment of the analyzer.

Indications of Deterioration
Visible signs of microbial growth, gross turbidity, or precipitate in the Ceruloplasmin reagents may indicate degradation and warrant discontinuation of use.

Specimen Collection and Preparation
Serum, collected after centrifugation of a clotted fasting blood specimen and free from hemolysis, is the recommended specimen. Avoid highly lipemic samples, which may produce excessively high scatter signals. Heparinized plasma can also be used for a specimen.

Sample Storage and Stability
Serum should be refrigerated at 2 - 8°C and can be used within 3 days. These samples can be stored frozen for up to 4 weeks.3

Interfering Substances
Results of studies conducted* show that the following substances may interfere with this Ceruloplasmin 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 1,000 mg/dL Intralipid*

* Intralipid, manufactured by KabiVitrum 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* 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
Ceruloplasmin Reagent
Ceruloplasmin

Materials required but not provided
Serum Protein Multi-calibrator 2 (Cat # ODR3023)

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

Calibration
The frequency of calibration for the Ceruloplasmin turbidimetric procedure is every 14 days. Calibration of this Ceruloplasmin procedure is accomplished by use of the Serum Protein Multi-calibrator 2 (Cat # ODR3023), which is traceable to IFCC International Reference Preparation CRM470 (RPPHS).

Recalibration of this test is required when any of these conditions exist:
1. A reagent lot number has changed or there is an observed shift in control values.
2. Major preventative maintenance was performed on the analyzer.
3. A critical part was replaced.

Quality Control
During operation of the Beckman Coulter AU analyzer, at least two levels of immunology 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 mg/L at 37°C.

Dynamic Range
The Ceruloplasmin turbidimetric procedure is linear from 60 – 2000 mg/L. 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.

Note: Samples from patients with abnormal lipoprotein metabolism such as those seen in cholecystitis or obstructive liver disease may give artificially negative Ceruloplasmin results. These samples are characterized by having extremely elevated Cholesterol values (> 387 mg/dL) and elevated Bilirubin. Such samples should be diluted 1 part sample to 4 parts deionized water prior to analysis and the result multiplied by 5.

Expected Values
Adults: 180 – 580 mg/L
This reference range was determined from healthy blood donors (data on file). 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 Ceruloplasmin reagent on Beckman Coulter analyzers according to established procedures. Results obtained in individual laboratories may differ.

Precision
Estimates of precision, based on CLSI recommendations, are consistent with typical performance. The within run precision is less than 5% CV and total precision is less than 10% CV. Assays of serum pools and control sera were performed and the data reduced following CLSI guidelines above.

<table>
<thead>
<tr>
<th>N = 80</th>
<th>Within run</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean, mg/L</td>
<td>SD</td>
<td>CV%</td>
</tr>
<tr>
<td>120.3</td>
<td>1.10</td>
<td>0.94</td>
</tr>
<tr>
<td>934.1</td>
<td>11.80</td>
<td>1.27</td>
</tr>
<tr>
<td>1810.5</td>
<td>17.10</td>
<td>0.94</td>
</tr>
</tbody>
</table>

Method Comparison
Patient samples were used to compare this Ceruloplasmin Reagent. The table below demonstrates representative performance on AU analyzers.

<table>
<thead>
<tr>
<th>Y Method</th>
<th>AU640</th>
</tr>
</thead>
<tbody>
<tr>
<td>X Method</td>
<td>Method 2</td>
</tr>
<tr>
<td>Slope</td>
<td>1.020</td>
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<tr>
<td>Intercept</td>
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<tr>
<td>Correlation Coeff. (r)</td>
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<tr>
<td>No. of Samples (n)</td>
<td>50</td>
</tr>
<tr>
<td>Range (mg/L)</td>
<td>90.0 - 510.0</td>
</tr>
</tbody>
</table>

References
6. Beckman Coulter Inc. data on samples collected from 80 blood donors in North America.
8. Data is on file for specific AU analyzers.

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