**Intended Use**
System reagent for the quantitative determination of Complement 4 (C4) in human serum on Beckman Coulter AU analyzers.

**Summary**
The Complement system is composed of a series of circulating blood proteins that serve as mediators of the inflammatory response. C4 is utilized only by the classical complement pathway, so that it is decreased only when this pathway is activated. In diseases activating the alternate pathway alone, C4 levels will be normal.\(^1\) Measurements of this protein aids in the diagnosis of immunologic disorders, especially those associated with deficiencies of complement components.

**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 AU400/400\(^e\)/480, AU600/640/640\(^e\)/680 and AU2700/5400 Beckman Coulter Analyzers.

**Reagents**
Final concentration of reactive ingredients:
- Tris buffer (pH 7.2) \(62 \text{ mmol/L}\)
- Polyethylene glycol 6000 \(1.6\% \text{ w/v}\)
- Goat anti-C4 antisera

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 C4 reagent is ready for 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, turbidity, precipitate or change in color in the C4 reagents may indicate degradation and warrant discontinuation of use.

**Specimen Collection and Preparation**
Serum is the recommended specimen. Prior to centrifugation, allow the sample to clot 15 – 30 minutes at room temperature, then refrigerate the sample at 2 - 8°C and allow clotting to continue for 30 – 60 minutes.\(^1\) To separate serum: rim the clot and centrifuge in refrigerated centrifuge. Serum should then be separated into multiple aliquots and frozen \((\leq -20^\circ\text{C})\) to avoid thawing and refreezing.

If cryoprecipitating antibodies are suspected, clot formation and centrifugation should proceed at 37°C as a complement fixation may occur if the specimen is chilled. In certain sera, chilling will significantly lower complement titer.\(^2\)

**Sample Storage and Stability**
Serum samples can be stored at 2 - 8°C for up to 8 days and at -20°C for up to 3 months without any loss of activity. Samples should be refrigerated or frozen if the assay cannot be run within 4 hours.\(^3\)

**Interfering Substances**
Results of studies\(^*\) show that the following substances may interfere with this C4 procedure:

<table>
<thead>
<tr>
<th>Substance</th>
<th>Interference</th>
<th>Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bilirubin</td>
<td>No significant interference up to 40 mg/dL</td>
<td>within 10%</td>
</tr>
<tr>
<td>Hemolysis</td>
<td>No significant interference up to 500 mg/dL</td>
<td>within 10%</td>
</tr>
<tr>
<td>Lipemia</td>
<td>No significant interference up to 500 mg/dL</td>
<td>within 10%</td>
</tr>
<tr>
<td>RF positive</td>
<td>No significant interference up to 15 – 1838 IU/mL</td>
<td>within 10%</td>
</tr>
</tbody>
</table>

\(^*\) 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\(^*\) for a compilation of reported interferences with this test.
C4

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

**Materials Provided**
C4 Reagent

**Materials required but not provided**
Serum Protein Multi-Calibrator (Cat # ODR3021).
MC Cal A (Cat # ODR30037) for Mastercurve enabled systems.

**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 C4 turbidimetric procedure is every 90 days. Calibration of this C4 procedure is accomplished by use of the Serum Protein Multi-Calibrator (Cat # ODR3021), 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 an appropriate Immunology control should be tested a minimum of once a day. In addition, these 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/dL at 37°C.

**Dynamic Range**
The C4 turbidimetric procedure is linear from 8 – 150 mg/dL. 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**
Serum:* 19 - 52 mg/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 C4 reagent on Beckman Coulter AU 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 3% CV and the total precision is less than 5% CV. Assays of serum pools and control sera were performed and the data reduced following CLSI guidelines above.

<table>
<thead>
<tr>
<th>N=100</th>
<th>Within run</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean, mg/dL</td>
<td>SD</td>
</tr>
<tr>
<td>15.1</td>
<td>0.4</td>
<td>2.3</td>
</tr>
<tr>
<td>27.8</td>
<td>0.3</td>
<td>1.2</td>
</tr>
</tbody>
</table>

**Method Comparison**
Patient samples were used to compare this C4 Reagent. The table below demonstrates representative performance on AU analyzers.

<table>
<thead>
<tr>
<th>T Method</th>
<th>X Method</th>
<th>Slope</th>
<th>Intercept</th>
<th>Correlation Coeff. (r)</th>
<th>No. of Samples (n)</th>
<th>Range (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AU640</td>
<td>AU600</td>
<td>0.96</td>
<td>1.1</td>
<td>0.9988</td>
<td>164</td>
<td>10 – 96</td>
</tr>
</tbody>
</table>

**References**
6. Beckman Coulter Inc., data on samples collected from 200 blood donors in North Texas.
8. Data is on file for specific AU analyzers.

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