Complement C3
For the Quantitative Determination of Human Complement C3 in Serum
Cat. No. KAI-009

INTENDED USE
For the quantitative determination of human complement C3 (3rd complement component) in serum by immunoturbidimetric assay. Complement C3 is a group of serum proteins that destroy infectious agents. Measurement of these proteins aids in the diagnosis of immunological disorders, especially those associated with deficiencies of complement components. FOR IN VITRO DIAGNOSTIC USE.

INTRODUCTION AND SUMMARY
C3 is the third complement component. It is one of a group of serum proteins that are active in the body's immune response. Complement C3 is an opsonic protein, playing a role in destroying infectious agents. The level of the 3rd complement component (C3) in serum can be used to help identify immunological disorders, especially those associated with deficiencies of complement components.1,2 Complement C3 has been measured using a variety of methods, including radioimmunoassay (RIA), radial diffusion, nephelometric, and enzyme-linked immunosorbent assay.2 The K-ASSAY® Complement C3 assay uses an immunoturbidimetric assay format.

PRINCIPLE OF TEST
The K-ASSAY® Complement C3 assay quantifies the 3rd complement component in the patient’s serum based by immunoturbidimetric assay. Calibrators, controls, and patient samples are pipetted into sample cups. Microvolumes of samples and antibody diluent are automatically pipetted into individual cuvettes. Following an initial incubation and measurement of sample blank, antisera is added to the cuvettes. The samples (antigen) and antisera are then mixed in the reaction cuvettes.

STORAGE AND HANDLING
All reagents should be stored refrigerated (2-8°C). Return reagents to 2-8°C promptly after use. Unopened reagents can be used for up to 18 months from the date of manufacture, as indicated by the expiration date on the package and bottle labels.

The K-ASSAY® Complement C3 assay can be run using a two-reagent clinical chemistry analyzer. Six calibrators are provided in the K-ASSAY® Multi-Analyte Calibrator. These calibrators are used to prepare a calibration curve for quantifying the levels of complement C3 present in the patient’s serum sample.

KIT COMPOSITION
Reagents (Liquid Stable)
R1: Buffer Reagent 4 x 20 mL Tris(hydroxymethyl)aminomethane
R2: Antiserum Reagent 2 x 10 mL Anti-human complement C3 goat antiserum (35%)

WARNINGS AND PRECAUTIONS
FOR IN VITRO DIAGNOSTIC USE. Rx only.
Not to be used internally in humans or animals. Normal precautions exercised in handling laboratory reagents should be followed.
Do not mix or use reagents from one test kit with those from a different lot number.
Do not use reagents past their expiration date stated on each reagent container label.
Do not pipette by mouth. Avoid ingestion and contact with skin.
Reagents in this kit contain sodium azide as a preservative. Sodium azide may form explosive amounts of water. For further information, refer to “Decontamination of Laboratory Sink Drains to Remove Azide Salts,” in the Manual Guide-Safety Management No. CDC-22 issued by the Centers for Disease Control, Atlanta, Georgia.

REAGENT STABILITY
Opened reagents can be used for 1 month if stored at 2-8°C. Discard reagents if they become contaminated. Evidence of cloudiness or particulate matter in solution is cause to discard.

INSTRUMENT
Measurement of absorbance is to be made with an instrument able to accurately read absorbance at 600 nm. Refer to the instrument manual from the manufacturer regarding the following:

a) Use or function
b) Installation procedures and requirements
c) Principles of operation
d) Performance characteristics, operating instructions
e) Calibration procedures including materials and/or equipment to be used
f) Operational precautions, limitations, and hazards
g) Service and maintenance information

SPECIMEN COLLECTION AND PREPARATION
It is recommended that specimen collection be carried out in accordance with NCCLS document M29-T2. No method can offer complete assurance that human blood samples will not transmit infection. Therefore, all blood samples should be considered potentially infectious.

Serum is required for this assay. Soon after the blood is drawn, it should be allowed to clot, centrifuged, and the serum separated from the clot to a plastic tube (not glass) within 2 hours. Serum should be stored refrigerated (2-8°C) and can be used within 1 week or should be stored frozen for up to 2 months.

Use plastic tubes for storing the sample, do not use glass.

AUTOMATED ANALYZER APPLICATION
Suitable for two-reagent automated analyzers that use a multi-point calibration method.

PROCEDURE
Materials Supplied
Reagent 1 (R-1) Buffer Reagent 4 x 20 mL
Reagent 2 (R-2) Antiserum Reagent 2 x 10 mL

Materials Required But Not Supplied
Calibrators: K-ASSAY® Multi-Analyte Calibrator, Cat. No. KAI-016C. (6 calibrators containing human serum with known levels of complement C3).

Two Reagent Clinical Chemistry Analyzer:
Capable of accurate absorbance readings at 600 nm
Capable of accurately dispensing the required volumes
Capable of maintaining 37°C

Reagent Procedure
Note: Allow all reagents and specimens to warm to room temperature. Mix all reagents gently before using.

An example of automated application (Hitachi 717): Sample ↓
1) ← R1 (Buffer Reagent) 250 µL
2) 37 °C, 5 min.
1) ← R2 (Antiserum Reagent) 70 µL
3) 37 °C, 5 min.
2) 2-point endpoint, 600 nm

Automated Method (Example)
Chemistry Parameters for Automatic Analyzer

<table>
<thead>
<tr>
<th>INSTRUMENT</th>
<th>Roche / Hitachi 717</th>
</tr>
</thead>
<tbody>
<tr>
<td>TEMPERATURE</td>
<td>37°C</td>
</tr>
<tr>
<td>TEST</td>
<td>C3</td>
</tr>
<tr>
<td>ASSAY CODE</td>
<td>(2 POINT) : (24) - (50)</td>
</tr>
<tr>
<td>SAMPLE VOLUME</td>
<td>(.5) (.)</td>
</tr>
<tr>
<td>R-1 VOLUME</td>
<td>(250) (.) (NO)</td>
</tr>
<tr>
<td>R-2 VOLUME</td>
<td>(.70) (.) (NO)</td>
</tr>
<tr>
<td>WAVELENGTH</td>
<td>(.600)</td>
</tr>
<tr>
<td>CALIB. METHOD</td>
<td>(NONLINEAR) (1) (6)</td>
</tr>
<tr>
<td>STD.(1) Conc.-POS.</td>
<td>(.1) (1)</td>
</tr>
<tr>
<td>STD.(2) Conc.-POS.</td>
<td>(.2) (2)</td>
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<tr>
<td>STD.(3) Conc.-POS.</td>
<td>(.3) (3)</td>
</tr>
<tr>
<td>STD.(4) Conc.-POS.</td>
<td>(.4) (4)</td>
</tr>
<tr>
<td>STD.(5) Conc.-POS.</td>
<td>(.5) (5)</td>
</tr>
<tr>
<td>STD.(6) Conc.-POS.</td>
<td>(.6) (6)</td>
</tr>
<tr>
<td>SD LIMIT</td>
<td>(999)</td>
</tr>
<tr>
<td>DUPLICATE LIMIT</td>
<td>(10000)</td>
</tr>
<tr>
<td>SENSITIVITY LIMIT</td>
<td>(0)</td>
</tr>
<tr>
<td>ABS. LIMIT (SLOPE)</td>
<td>(32000) (INCREASE)</td>
</tr>
<tr>
<td>PROZONE LIMIT</td>
<td>(-32000) (LOWER)</td>
</tr>
<tr>
<td>EXPECTED VALUE</td>
<td>(-99999) (99999)</td>
</tr>
<tr>
<td>PANIC VALUE</td>
<td>(-99999) (99999)</td>
</tr>
<tr>
<td>INSTRUMENT FACTOR</td>
<td>(.100)</td>
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</tbody>
</table>

*1-6: Input concentration of calibrators.

Parameters for other automated analyzers are available.

CALIBRATION
It is recommended that complement C3 levels be determined using a multi-point calibration curve prepared using the K-ASSAY® Multi-Analyte Calibrator. It is recommended that the user determine calibration curve frequency as this depends on the instrument and type/number of other assays being performed. Initially, calibration should be performed each day.

QUALITY CONTROL
Normal and abnormal controls of known concentration should be included in each assay performed. These controls should fall within the stated values assigned to the controls. The validity of the assay is in question if the value for the controls generated by the assay's calibration
Complement C3 levels are determined using the prepared calibration curve.

**LIMITATIONS OF PROCEDURE**

The measurable range for complement C3 is between 30 - 350 mg/dL. Grossly lipemic samples and samples with very high triglyceride concentrations should be diluted in part sample with 1 part isotonic saline or filtered to decrease nonspecific light scattering. Multiply results by 2 to compensate for the dilution. 

If the complement C3 concentration of a patient sample is greater than the highest calibrator value, dilute 1 part sample with 1 part isotonic saline or filtered to compensate for the dilution.

**PERFORMANCE**

**Precision**

The precision for the K-ASSAY® Complement C3 assay was determined using packaged reagents, pooled human serum, and a Hitachi 717 analyzer.

**Sample I**

<table>
<thead>
<tr>
<th>Sample</th>
<th>N = 20</th>
<th>Mean = 33.7</th>
<th>SD = 1.12</th>
<th>CV = 3.33%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample II</td>
<td>N = 20</td>
<td>Mean = 75.6</td>
<td>SD = 1.64</td>
<td>CV = 2.22%</td>
</tr>
<tr>
<td>Sample III</td>
<td>N = 20</td>
<td>Mean = 112.4</td>
<td>SD = 2.18</td>
<td>CV = 1.94%</td>
</tr>
</tbody>
</table>

**Sample II**

<table>
<thead>
<tr>
<th>Sample</th>
<th>N = 10</th>
<th>Mean = 35.4</th>
<th>SD = 1.29</th>
<th>CV = 3.63%</th>
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</thead>
<tbody>
<tr>
<td>Sample III</td>
<td>N = 10</td>
<td>Mean = 84.3</td>
<td>SD = 1.95</td>
<td>CV = 2.31%</td>
</tr>
</tbody>
</table>

**Accuracy / Correlation**

A comparison of the K-ASSAY® Complement C3 assay and an INCSTAR Complement C3 Test Kit was performed using a Hitachi 717. The test results provided the following data:

\[ y = 0.842x + 4.727 \]

\[ r = 0.925 \]

\[ n = 60 \]

\[ x = \text{INCSTAR Complement C3 Test Kit} \]

\[ y = \text{K-ASSAY® Complement C3 assay} \]

<table>
<thead>
<tr>
<th>x min</th>
<th>y min</th>
<th>max</th>
<th>mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>78</td>
<td>74</td>
<td>188</td>
<td>171</td>
</tr>
<tr>
<td>135</td>
<td>118</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**INTERERENCE**

- Bilirubin C: No interference up to 20 mg/dL
- Bilirubin F: No interference up to 20 mg/dL
- Hemoglobin: No interference up to 500 mg/dL
- Intralipid: No interference up to 500 mg/dL

**EXPECTED VALUE**

The expected value as reported is between 74 - 148 mg/dL. Each laboratory should establish its own expected values using this kit.

**REFERENCES**


**EXPECTED VALUE**

- Complement C3: Within Run
  - CV = 1.48%
  - SD = 1.70
  - Mean = 114.4
  - N = 10

- Complement C3: Within Run
  - CV = 1.94%
  - SD = 2.18
  - Mean = 112.4
  - N = 20

- Complement C3: Between Runs
  - CV = 2.84
  - SD = 3.19
  - Mean = 135.7
  - N = 10

**EU AUTHORIZED REPRESENTATIVE**

Advena Ltd.
Tower Business Centre, 2nd Flr.,
Tower Street, Swatar, BKR 4013 Malta

**ORDERING / PRICING / TECHNICAL INFORMATION**

KAMYA BIO MEDICAL COMPANY
12779 Gateway Drive
Seattle, WA 98168 USA
TEL: (206) 575-8068 / (800) 526-4925
FAX: (206) 575-8094

**LABELING SYMBOLS**

<table>
<thead>
<tr>
<th>LOT</th>
<th>Lot Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>GT</td>
<td>Reagent</td>
</tr>
<tr>
<td>E</td>
<td>Expiration or “Use By” Date</td>
</tr>
<tr>
<td>Ref</td>
<td>Catalog Number</td>
</tr>
<tr>
<td>IVD</td>
<td>For In Vitro Diagnostics Use</td>
</tr>
<tr>
<td>M</td>
<td>Manufacturer</td>
</tr>
<tr>
<td>I</td>
<td>Consult Package Insert for Instructions for Use</td>
</tr>
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</table>

**K-ASSAY® Complement C3**

30 - 350 mg/dL