**K-ASSAY®**

**Complement C4**

For the Quantitative Determination of Human Complement C4 in Serum

[Cat. No. KAI-010]

**INTENDED USE**

For the quantitative determination of human complement C4 (4th complement component) in serum by immunoturbidimetric assay. Complement 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.

**IN VITRO DIAGNOSTIC USE.**

**INTRODUCTION AND SUMMARY**

C4 is the fourth complement component. It is one of a group of serum proteins that are active in the body's immune response to destroy infectious agents. Measurement of the level of the 4th complement component (C4) in serum can aid in the diagnosis of immunological disorders, especially those associated with deficiencies of complement components.

The **K-ASSAY®** Complement C4 assay is intended for the quantitative determination of human complement C4 by immunoturbidimetric assay. The antisera used in the kit was produced against purified human complement C4. The complement C4 antibody interacts with the complement C4 in the serum forming immune complexes. The immune complexes cause an increase in light scattering that correlates with the concentration of serum complement C4.

Complement C4 has been measured using a variety of methods, including radioimmunoassay (RIA), radial diffusion, nephelometric, and enzyme-linked immunosorbent assay. The **K-ASSAY®** Complement C4 assay uses an immunoturbidimetric assay format.

**PRINCIPLE OF TEST**

The **K-ASSAY®** Complement C4 assay quantifies the 4th complement component in the patient's serum based on 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, antigen is added to the cuvettes. The samples (antigen) and antisera are then mixed in the reaction cuvettes. Insoluble antigen-antibody (immune) complexes form. The immune complexes cause an increase in light scattering that correlates with the concentration of serum complement C4. Following an incubation period lasting approximately 5 min., the absorbance of the solution is measured at 600 nm.

A calibration curve is generated by assaying a series of calibrators with known concentrations of proteins and using the instrument's data reduction capability or manually plotting the change in absorbance versus concentration. Concentration of the control and patient samples are interpolated from the calibration curve. The antisera used in the kit is a goat polyclonal antibody specific for human complement C4.

The **K-ASSAY®** Complement C4 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 C4 present in the patient's serum sample.

**KIT COMPOSITION**

Reagents (Liquid Stable)

- R1: Buffer Reagent 4 x 20 mL
- R2: Antiserum Reagent 2 x 10 mL
- Anti-human complement C4 goat antisera (40%)

**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. Sodium azide is very toxic by ingestion, and there is a possibility of the formation of toxic aminomethanes. When disposing of reagents through plumbing fixtures, flush with copious amounts of water.

**STORAGE AND HANDLING**

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

**REAGENT STABILITY**

Opened reagents can be used for one month if stored at 2-8°C. Discard reagents if they become contaminated. Evidence of cloudiness or particulate material 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:

- Use or function
- Installation procedures and requirements
- Principles of operation
- Performance characteristics, operating instructions
- Calibration procedures including materials and / or equipment to be used
- Operational precautions, limitations, and hazards
- 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 one 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 two-point and 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 C4).

**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

**ASSAY CODE**

(2 POINT) : (24) - (50) SAMPLE VOLUME (5) ( ) WAVELENGTH (4) µ ( ) 600 CALIB. METHOD (NONLINEAR) (1) (6) STD.(1) Conc.-POS. (1)” - (1) STD.(2) Conc.-POS. (2)” - (2) STD.(3) Conc.-POS. (3)” - (3) STD.(4) Conc.-POS. (4)” - (4) STD.(5) Conc.-POS. (5)” - (5) STD.(6) Conc.-POS. (6)” - (6) SD LIMIT (9999)

**DIAGNOSTIC USE.**

**R1 VOLUME (250) ( ) R2 VOLUME (70) ( ) R3 VOLUME (75) ( ) R4 VOLUME (125) ( ) R5 VOLUME (130) ( ) R6 VOLUME (135) ( )**

**ASSAY 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**

- 5 µL

**Chemistry Parameters for Automatic Analyzer**

**INSTRUMENT**

Hitachi 717

**TEMPERATURE**

37°C

**TEST**

- C4

**ASSAY CODE**

- (2 POINT) : (24) - (50)

**SAMPLE VOLUME**

- (5) µL

**R1 VOLUME**

- (250) µL

**R2 VOLUME**

- (70) µL

**R3 VOLUME**

- (75) µL

**R4 VOLUME**

- (125) µL

**R5 VOLUME**

- (130) µL

**R6 VOLUME**

- (135) µL

**WAVELENGTH**

- ( ) 600

**CALIB. METHOD**

- (NONLINEAR) (1) (6)

**STD.(1) Conc.-POS.**

- (*) - (1) STD.(2) Conc.-POS.**

- (*) - (2) STD.(3) Conc.-POS.**

- (*) - (3) STD.(4) Conc.-POS.**

- (*) - (4) STD.(5) Conc.-POS.**

- (*) - (5) STD.(6) Conc.-POS.**

- (*) - (6)

**SD LIMIT**

- (9999)

**DUPLICATE LIMIT**

- (10000)

**SENSITIVITY LIMIT**

- (0)

**ABS. LIMIT (SLOPE)**

- (32000) (INCREASE)

**PROZONE LIMIT**

- (32000) (LOWER)

**EXPECTED VALUE**

- (-) (-9999) (99999)

**PANIC VALUE**

- (-) (-9999) (99999)

**INSTRUMENT FACTOR**

- (1.00)

* 1-6 Input concentration of calibrators

Parameters for other automated analyzers are available.

**CALIBRATION**

It is recommended that complement C4 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.

**RESULTS / CALCULATIONS**

Complement C4 levels are determined using the prepared calibration curve.
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 curve does not fall within the stated range. Recalibrate if the value determined for the controls falls outside the stated recovery range.

LIMITATIONS

The measurable range for complement C4 is between 8 and 80 mg/dL. Grossly lipemic samples and samples with very high triglyceride concentrations should be diluted 1 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 C4 concentration of a patient sample is greater than the highest calibrator value, dilute 1 part sample with 4 parts isotonic saline and reassay. Multiply results by 5 to compensate for the dilution.

The absorbance of isotonic saline should be below 0.05 and the absorbance of the 39 mg/dL calibrator should be between 0.05 and 0.15 after allowing for the reagent blank.

PERFORMANCE

Precision

The precision for the K-ASSAY® Complement C4 assay was determined using packaged reagents, pooled human serum, and a Hitachi 717 analyzer.

<table>
<thead>
<tr>
<th>Sample</th>
<th>N</th>
<th>Mean</th>
<th>SD</th>
<th>CV</th>
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<tbody>
<tr>
<td>I</td>
<td>20</td>
<td>5.7</td>
<td>0.29</td>
<td>5.17%</td>
</tr>
<tr>
<td>II</td>
<td>20</td>
<td>36.3</td>
<td>0.76</td>
<td>2.1%</td>
</tr>
<tr>
<td>III</td>
<td>20</td>
<td>58.6</td>
<td>1.98</td>
<td>3.38%</td>
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</table>

Accuracy / Correlation

A comparison of the K-ASSAY® Complement C4 assay and an Incstar Complement C4 Test Kit was performed using a Hitachi 717. The test results provided the following data:

*y* = 0.7399x + 2.46

r = 0.998
n = 60

x = Incstar Complement C4 Test Kit
y = K-ASSAY® Complement C4 assay

<table>
<thead>
<tr>
<th>x_min</th>
<th>y_min</th>
<th>x_max</th>
<th>y_max</th>
<th>Mean x</th>
<th>Mean y</th>
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</thead>
<tbody>
<tr>
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<td>9</td>
<td>56</td>
<td>45</td>
<td>31</td>
<td>25</td>
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EXPECTED VALUES

The expected value as reported is between 14 and 39 mg/dL. Each laboratory should establish its own expected values using this kit.

REFERENCES


LABELING SYMBOLS

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Description</th>
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<tbody>
<tr>
<td>Lot Number</td>
<td></td>
</tr>
<tr>
<td>Reagent</td>
<td></td>
</tr>
<tr>
<td>Expiration or “Use By” Date</td>
<td></td>
</tr>
<tr>
<td>Catalog Number</td>
<td></td>
</tr>
<tr>
<td>For In Vitro Diagnostic Use</td>
<td></td>
</tr>
<tr>
<td>Temperature Limitation. Store between 2 and 8 degrees C</td>
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</tr>
<tr>
<td>Potential Human Biohazard</td>
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<tr>
<td>Manufacturer</td>
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<td>Consult Package Insert for Instructions for Use</td>
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<td>Authorized Representative in the European Community</td>
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