K-ASSAY® IgM
For the Quantitative Determination of IgM in Serum
Cat. No. KAI-015

INTENDED USE
For the quantitative determination of human IgM in serum by immunoturbidimetric assay. Measurement of IgM aids in the diagnosis of abnormal protein metabolism and the body's lack of ability to resist infectious agents. FOR IN VITRO DIAGNOSTIC USE.

INTRODUCTION AND SUMMARY
Immunoglobulins are an important part of the body's immune response. The immunoglobulin M (IgM) molecule is composed of a pentamer of two light chains (kappa and lambda) and the two mu heavy chains plus a J chain. IgM is the largest immunoglobulin molecule, MW = 900,000 daltons. It makes up approximately 6% of the total immunoglobulin. IgM is the body's primary immune response.

The level of IgM in serum increases in hepatic disease, and bacterial or viral infections. The levels of IgM decrease with congenital immunodeficiency. The measurement of IgM provides useful information in the assessment of these diseases or conditions.

IgM levels in serum may be quantified using a variety of methods such as turbidimetric, nephelometric, immunodiffusion, or immunoblot. The immunoturbidimetric method, taking advantage of the light scattering properties of the antigen-antibody complexes, Antibody will bind specifically to the antigen in question, forming a complex. This complex can be quantified by measuring light absorption at 700 nm. The sensitivity and the rate of forming the immune-complex can be increased by the addition of polyethylene glycol (PEG). PRINCIPLE OF TEST

Human serum, containing IgM, is diluted with buffer containing polyethylene glycol (PEG) and mixed with specific polyclonal goat anti-IgM antisera. The antigen (IgM) and the specific goat antibody form complexes. The formation of the complexes is accelerated and enhanced by PEG. This allows for the reaction to rapidly reach its endpoint with greater sensitivity and less concern for false negative values due to antigen excess. The immune complexes cause an increase in light scattering that correlates with the concentration of IgM in the serum. Light scattering is measured by reading turbidity at 340 nm and 700 nm. Six calibrators in the K-ASSAY® Multi-Analyte Calibrator are to be used to prepare a calibration curve for quantifying the levels of IgM present in the patient sample.

KIT COMPOSITION
Reagents (Liquid Stable)
R1: Buffer Reagent, pH 7.6 4 x 20 mL
Tris(hydroxymethyl)aminomethane (100mM)
R2: Antiserum Reagent, pH 7.6 2 x 10 mL
Anti-human IgM goat antiserum (30%)

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.

As part of this kit contain sodium azide as a
preservative. Sodium azide may form explosive
compounds in metal drain lines. When disposing of
reagents through plumbing fixtures, flush with copious
amounts of water. For further information, refer to
“Decontamination of Laboratory Sink Drains to Remove

PRINCIPLE OF TEST

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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 used for up to 18 months from the date of
manufacture, as indicated by the expiration date on the
package and bottle labels.

REAGENT STABILITY
Open reagents can be used for 1 month if stored at
2-8°C. Discard reagents if they become contaminated.
Evidence of cloudiness or particulate material in solution is
cardiscard.

INSTRUMENT
Measurement of absorbance is to be made with an
instrument able to accurately read absorbance at 340 and
700 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

ASSAY

Sample 5 μL
↓
\( R_1 \) (Buffer Reagent) 250 μL
\( R_2 \) (Antiserum Reagent) 70 μL
↓
37 °C, 5 min.

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>IgM</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>(700) (340)</td>
</tr>
<tr>
<td>CALIB. METHOD</td>
<td>(NONLINEAR) (1) (6)</td>
</tr>
<tr>
<td>STD.(1) Conc.-POS.</td>
<td>(1) ( )</td>
</tr>
<tr>
<td>STD.(2) Conc.-POS.</td>
<td>(2)</td>
</tr>
<tr>
<td>STD.(3) Conc.-POS.</td>
<td>(3) ( )</td>
</tr>
<tr>
<td>STD.(4) Conc.-POS.</td>
<td>(4) ( )</td>
</tr>
<tr>
<td>STD.(5) Conc.-POS.</td>
<td>(5) ( )</td>
</tr>
<tr>
<td>STD.(6) Conc.-POS.</td>
<td>(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>(320000) ( LOWER )</td>
</tr>
<tr>
<td>EXPECTED VALUE</td>
<td>(-999999) (999999)</td>
</tr>
<tr>
<td>PANIC VALUE</td>
<td>(-999999) (999999)</td>
</tr>
<tr>
<td>INSTRUMENT FACTOR</td>
<td>(1.00)</td>
</tr>
</tbody>
</table>

1-6: Input concentration of calibrators.

Parameters for other automated analyzers are available.

CALIBRATION
It is recommended that IgM 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.

K-ASSAY® IgM

KAMIYA BIO MEDICAL COMPANY

Rev. 2019-01-25
QUALITY CONTROL

A quality control program is recommended for all clinical testing and laboratories. It is recommended that control sera, both normal and abnormal, be run with each batch of samples to monitor the procedure.

The values obtained for controls should fall within the manufacturer’s specified range. A laboratory may establish its own control serum by assaying the serum a sufficient number of times to generate a valid mean and acceptable range.

RESULTS / CALCULATIONS

IgM levels are determined using the prepared calibration curve.

LIMITATIONS OF PROCEDURE

The measurable range for this IgM test kit is between 10 - 350 mg/dL.

If IgM concentrations are greater than highest calibrator value, dilute 1 part sample with 4 parts isotonic saline and re-assay. Multiply the result by 5 to compensate for the dilution.

PERFORMANCE

Precision Assay: Within Run

<table>
<thead>
<tr>
<th>Sample I</th>
<th>Sample II</th>
<th>Sample III</th>
</tr>
</thead>
<tbody>
<tr>
<td>N = 20</td>
<td>N = 20</td>
<td>N = 20</td>
</tr>
<tr>
<td>Mean = 69</td>
<td>Mean = 138</td>
<td>Mean = 207</td>
</tr>
<tr>
<td>SD = 0.64</td>
<td>SD = 1.11</td>
<td>SD = 1.35</td>
</tr>
<tr>
<td>CV = 0.9%</td>
<td>CV = 0.8%</td>
<td>CV = 0.7%</td>
</tr>
</tbody>
</table>

Precision Assay: Between Runs

<table>
<thead>
<tr>
<th>Sample I</th>
<th>Sample II</th>
<th>Sample III</th>
</tr>
</thead>
<tbody>
<tr>
<td>N = 7</td>
<td>N = 7</td>
<td>N = 7</td>
</tr>
<tr>
<td>Mean = 94</td>
<td>Mean = 183</td>
<td>Mean = 345</td>
</tr>
<tr>
<td>SD = 1.4</td>
<td>SD = 0.9</td>
<td>SD = 3.5</td>
</tr>
<tr>
<td>CV = 1.5%</td>
<td>CV = 0.5%</td>
<td>CV = 1.0%</td>
</tr>
</tbody>
</table>

Accuracy / Correlation

A comparison of the K-ASSAY® IgM kit and an INCSTAR IgM Test Kit was performed on a Hitachi 704 automated analyzer and a COBAS Mira. The test results provided the following data:

\[
y = 1.0927x - 0.71 \\
\text{r} = 0.98835 \\
n = 45 \\
x = \text{INCSTAR IgM Test Kit} \\
y = \text{K-ASSAY® IgM assay} \\
\]

Assay Range

10 - 350 mg/dL

INTERFERENCE

- Bilirubin: 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 is between 52 - 217 mg/dL. These values were determined using normal serum from 90 healthy donors. Each laboratory should establish its own expected values using this kit.

REFERENCES


LABELING SYMBOLS

- LOT: Lot Number
- REF: Catalog Number
- IVD: For In Vitro Diagnostics Use
- TEMP: 2-8 °C
- Manufacturer
- Authorized Representative in the European Community

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ORDERING / PRICING / TECHNICAL INFORMATION