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 immunoassay.[1,2,3,4] This kit uses an immunoturbidimetric method, taking advantage of the light scattering properties of the antigen-antibody complexes.[5] 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).[6]

PRINCIPLE OF TEST

Human serum, containing IgM, is diluted with buffer containing polyethylene glycol (PEG) and mixed with specific polyclonal goat anti-IgM antiserum. 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.

Reagents in 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 Azide Salts," in the Manual Guide-Safety Management No. CDC-22 issued by the Centers for Disease Control, Atlanta, Georgia.

REAGENT PREPARATION

The reagents are ready to use. They do not need to be reconstituted.

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

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 material in solution is cause to discard.

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 can offer complete assurance that human blood samples will not transmit infection. Therefore, all blood samples should be considered potentially infectious.

After drawing blood, allow it to completely coagulate. Centrifuge the coagulated blood and collect the supernatant. The supernatant can be directly used for testing without dilution. Samples may be stored for up to 1 week refrigerated. Serum samples stored for extended periods should be frozen at -20°C.

For storage of samples for more than a few days, use of plastic tubes is recommended instead of 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 IgM).

Two Reagent Clinical Chemistry Analyzer:
Capable of accurate absorbance readings at 340 / 700 nm
Capable of accurately dispensing the required volumes
Capable of maintaining 37°C

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
↓
• ← R1 (Buffer Reagent) — 250 μL
↓ 37 °C, 5 min.
• ← R2 (Antiserum Reagent) — 70 μL
↓ 37 °C, 5 min.
2-point endpoint, 340/700 nm

Automated Method (Example)

Chemistry Parameters for Automatic Analyzer

INSTRUMENT	Roche / Hitachi 717
TEMPERATURE	37°C
TEST	(IgM)
ASSAY CODE	(2 POINT) : (24) - (50)
SAMPLE VOLUME	(5) ( )
R-1 VOLUME	(250) ( ) (NO)
R-2 VOLUME	(70) ( ) (NO)
WAVELENGTH	(700) (340)
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	(999)
DUPLICATE LIMIT	(10000)
SENSITIVITY LIMIT	(0)
ABS. LIMIT (SLOPE)	(32000) (INCREASE)
PROZONE LIMIT	(-320000) (LOWER)
EXPECTED VALUE	(-99999) (99999)
PANIC VALUE	(-99999) (99999)
INSTRUMENT FACTOR	(1.00)

*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.

QUALITY CONTROL

A quality control program is recommended for all clinical testing and laboratories. It is recommended that control serums, 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

Precision Assay: Within Run

Sample I	Sample II	Sample III
N = 20	N = 20	N = 20
Mean = 69	Mean = 138	Mean = 207
SD = 0.64	SD = 1.11	SD = 1.35
CV = 0.9%	CV = 0.8%	CV = 0.7%

Precision Assay: Between Runs

Sample I	Sample II	Sample III
N = 7	N = 7	N = 7
Mean = 94	Mean = 183	Mean = 345
SD = 1.4	SD = 0.9	SD = 3.5
CV = 1.5%	CV = 0.5%	CV = 1.0%

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
r = 0.98835
n = 45
x = INCSTAR IgM Test Kit
y = **K-*ASSAY*®** IgM assay

x min	= 23	y min	= 21
max	= 392	max	= 438
mean	= 138	mean	= 150

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

1. Sekiguchi, M., *et al*., Saishinkensa, 1:15, 1983.
2. Terashaki, S., *et al*., Kiki-Shiyaku, 6:728, 1983.
3. Bergstrom, K., *et al*. *Scand. J. Clin. Lab. Invest.*, 40:673, 1980.
4. Malkus, H., *et al. Clinica Chimica Acta*, 88:523-530, 1978.
5. Killingsworth, L.M. and J. Savory. *J. Clin. Chem*. 19:403-407, 1973.
6. Lizana, J. and K. Helling. *Clin. Chem.* 20:1181, 1974.

LABELING SYMBOLS

LOT	Lot Number
RGT	Reagent
	Expiration or "Use By" Date
REF	Catalog Number
IVD	For *In Vitro* Diagnostics Use
2-8 °C	Temperature Limitation. Store between 2 and 8 degrees C
	Manufacturer
	Consult Package Insert for Instructions for Use
EC REP	Authorized Representative in the European Community

EU AUTHORIZED REPRESENTATIVE

CE

EC REP

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