Pariksha Biotech
A game changer in IVD

IgM Estimation Kit

High-Q Immunoglobulin IgM

Turbidimetric Immuno Assay (TIA)

HIGH®
Command on Quality

Intended Use:

Diagnostic reagent for quantitative in vitro determination of immunoglobulin M (IgM) in serum on photometric systems

Summary

The human immunoglobulin classes (IgG, IgA, IgM, IgE and IgD) are a group of functionally and structurally closely related glycoproteins. Human IgM has a molecular weight of about 970 000 dalton and consists five Y-shaped molecules which are bound together by a joining peptide. Each of the five Y-shaped units consists of two identical heavy chains and two identical light chains which are bound together by disulfide bonds. IgM is produced by plasma cells (B-cells) and represents about 5% of all soluble immmunoglobulin classes. The main function of IgM is to bind to antigens, initiating complement activation and trigger further catabolism of the antigen. IgM is the immunoglobulin class synthesized first after initial contact with a new antigen.

Decreased IgM concentrations occur in primary as well as in secondary immunodeficiency syndromes. Increased loss of proteins due to severe inflammation of the bowel may result in a decreased IgM concentration. A high increase in one immunoglobulin class due to multiple myeloma may result in a

decrease in other Immunoglobulin classes like IgM.

Increased IgM concentrations can be observed in severe infections and autoimmune diseases. Many forms of Myeloma and especially Waldenström's macroglobulinemia, produce high amounts of monoclonal or polyclonal IgM. Quantitative IgM determination is necessary for differential diagnosis of these diseases.

All methods for IgM quantitation are calibrated for polyclonal IgM. The quantitation of monoclonal IgM is not standardized and values may differ for different reagents and methods. Values should only be used for follow up studies. Monoclonal immunoglobulinemia requires detailed differential diagnostic investigation in addition to the quantitative determination.

Principle

Determination of the IgM concentration by photometric measurement of antigen-antibody-reaction of antibodies to human IgM with IgM present in the sample.

Reagents

Components and Concentrations

R1:	TRIS	pH 7.5	100 mmol/L
	NaCl		150 mmol/L
R2:	TRIS	pH 8.0	100 mmol/L
	NaCl		1150 mmol/L
	Anti-human IgM antibody (goat)		< 1%

Storage Instructions and Reagent Stability

All the reagents are liquids and are stable till the expiry date mentioned on the labels when stored properly at 2-8°C

IgM Calibrator: Concentration lot specific and available as Liquid Calibrator Calibrators are stable till the expiry date mentioned on the labels when stored properly at 2-8°C

IgM Controls: Concentration lot specific and available as Lyophilized Controls and are available optionally. Controls are stable till the expiry date mentioned on the labels when stored properly at 2-8°C

Specimen:

Serum is the specimen

Stability : 7 Days at 2-8°C and1 month at –20°C when frozen as aliquots

Avoid Freeze Thaw cycles

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TEST PROCEDURE:

System Parameters:

Calibration Method	**Endpoint-Bichromatic**
Reaction Direction	Increasing
Primary Wave Length	340
Secondary Wave Length	700 (600-700)
Flow Cell Temp.	37°C
Blank	Reagent Blank
Reagent Volume	500 µl (R1+R2)
Sample Volume)	5 µl
Calibrators Conc	Lot Specific (Check the labels))
Units	mg/dL
Linearity	800

PROCEDURE:

Reagent	Reagent Blank	Cal	Sample
IgM R1 Reagent	400 µL	400 µL	400 µL
Calibrator	-------	5 µL	------
Sample	-------	------	5 µL
Incubate for 5 Minutes at 37°C			
IgM R2 Reagent	100 µL	100 µL	100 µL

Mix well and incubate for 5 minutes at 37°C Read the absorbance of Calibrator (C) Sample (S) against Reagent Blank (B) Bichromatically at 340 nms (Primary Wavelength) and 700 nms (Secondary Wavelength- (600-700 nms)

Calculations:

$$\text{IgM (in mg/dl)} = \frac{\text{Abs. of Sample}}{\text{Abs. of Calibrator}} \times \text{Calibrator Concentration}$$

Performance Characteristics

Measuring Range (Linearity)

The test has been developed to determine concentrations of IgM within a measuring range from 25 - 800 mg/dL, at least up to the concentration of the highest calibrator. When values exceed the upper range samples should be diluted 1 + 1 with NaCl solution (9 g/L) and the result multiplied by 2.

Specificity/Interferences

Due to its antibodies, High-Q Immunoglobulin M is a specific immunoassay for human IgM. No interference was observed by conjugated and unconjugated bilirubin up to 60 mg/dL, hemoglobin up to 1000 mg/dL, lipemia up to 2000 mg/dL triglycerides and RF up to 1700 IU/mL.

No cross reaction with IgG or IgA was observed under test conditions.

Sensitivity/Limit of Detection

The lower limit of detection is (the minimum concentration which can be measured and distinguished from zero) is 3 mg/dL.

Imprecision

According to protocol EP-5 of the NCCLS (National Committee of Clinical Laboratory Standards)

Within-run precision	Mean	SD	CV
n = 40	[mg/dL]	[mg/dL]	[%]
Sample 1	87.3	2.05	2.35
Sample 2	275	6.06	2.21
Sample 3	420	8.92	2.12
Between day precision	Mean	SD	CV
n = 40	[mg/dL]	[mg/dL]	[%]
Sample 1	87.3	1.78	2.04
Sample 2	275	3.43	1.25
Sample 3	420	7.12	1.69

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Method Comparison:

A comparison of High-Q Immunoglobulin M (y) with a nephelometric test (x) using 77 samples gave following results:

y = 0.93 x + 4.23 mg/dL; r = 0.992.

Reference Range:

Adults :		**40 – 230 mg/dL**
Children :	**Newborns**	**10 – 30 mg/dL**
	1 – 3 month(s)	**10 – 70 mg/dL**
	4 – 6 months	**20 – 100 mg/dL**
	7 – 12 months	**30 – 100 mg/dL**
	2 years	**40 – 140 mg/dL**
	3 – 5 years	**40 – 180 mg/dL**
	6 –9 years	**40 – 160 mg/dL**
	10 – 13 years	**40 – 150 mg/dL**

Each laboratory should check if the reference ranges are transferable to its own patient population and determine own reference ranges if necessary.

Literature:

1. Thomas L. Clinical Laboratory Diagnostics. 1st ed. Frankfurt: TH-Books Verlagsgesellschaft; 1998. p. 667-78.
2. Johnson AM, Rohlfs EM, Silverman LM. Proteins. In: Burtis CA, Ashwood ER. editors. Tietz textbook of clinical chemistry. 3rd ed. Philadelphia: W. B. Saunders Company; 1999. p. 507-12.
3. Bartl R, Hoechtlen-Vollmar W, Thomas L. Monoclonal immunoglobulins. In: Th omas L. Clinical Laboratory Diagnostics. 1st ed. Frankfurt: TH-Books Verlagsgesellschaft; 1998. p. 742-58.
4. Guder WG, Narayanan S et al. List of Analytes; Preanalytical Variables. 1st ed. Darmstadt: Git Verlag, 1996: 16-7.
5. Young DS. Effects of Drugs on Clinical Laboratory Tests. 5th ed. Volume 1 and 2. Washington, DC: The American Association for Clinical Chemistry Press 2000.
6. Dati F, Schumann G, Thomas L, Aguzzi F, Baudner S, Bienvenu J et al. Consensus of a group of professional societies and diagnostic companies on guidelines for interim reference ranges for 14 proteins in serum based on the standardization against the IFCC/BCR/CAP reference material (CRM 470). Eur J Clin Chem Clin Biochem 1996; 34: 517-20.
7. Heil R, Koberstein R, Zawta B. Referenzbereiche für Kinder und Erwachsene. Roche Diagnostics 2004. p. 48–49.
8. Bakker AJ, Mücke M. Gammopathy interference in clinical chemistry assays: Mechanism, detection and prevention. Clin Chem Lab Med 2007; 45(9): 1240–1243.

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Ordering Information

Ref./Cat. No.	Pack Size	Presentation
P-IgM-25	25 ml	Liquid Stable two Reagents and Liquid Calibartor

Product Features

- **Liquid Stable, Ready to use two reagents**
- **10 Minutes Endpoint-Bichrimatic Assay**
- **Liquid Stable Calibrator Provided**
- **Linearity: 25-800 mg/dL**
- **Measuring Wavelength - Primary 340 nm, Secondary 700 nms (600-700 nms)**
- **Serum is the Specimen**
- **Available as multipurpose reagents and dedicated system packs**

Symbols used with IVD devices

	Date of manufacture		Manufactured by
IVD	In vitro diagnostic device		Keep away from sunlight
	Do not freeze		This way up
	Use by (yyyy-mm-dd or mm/yyyy)	R	Reagent
CAL	Calibrator Material	LOT	Batch code
2°C 8°C	Temperature limitation (store at)	CONTROL	Control
	Consult instructions for use		Keep dry Keep away from rain
REF	Catalog Number		

eIFU Indicator

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SCAN TO EXPLORE MORE

Manufactured in India by :
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AN ISO 13485 Certified Company

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