

IgM Estimation Kit

High-Q Immunoglobulin IgM





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 antigenantibody-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 $2-8^{\circ}\text{C}$

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

lgM 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 $2-8\,^{\circ}\text{C}$

Specimen:

Serum is the specimen

Stability: 7 Days at $2-8^{\circ}$ C and 1 month at -20° C when frozen as aliquots Avoid Freeze Thaw cycles



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 uL | 100 uL | 100 uL | | | | | |

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:

Performance Characteristics Measuring Range (Linearity)

The test has been developed to determine concentrations of lgM 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 $3\,\text{mg/dL}$.

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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Turbidimetric Immuno Assay (TIA)



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: 10-30 mg/dL Newborns 1-3 month(s) 10-70 mg/dL 20-100 mg/dL 4-6 months 7-12 months 30-100 mg/dL 2 years 40-140 mg/dL 40-180 mg/dL 3-5 years 40-160 mg/dL 6 -9 years

10-13 years

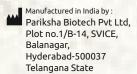
40-150 mg/dL Each laboratory should check if the reference ranges are transferable to its own patient population and determine own reference ranges if necessary.

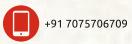
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- Heil R, Koberstein R, Zawta B. Referenzbereiche für Kinder und Erwachsene. Roche Diagnostics 2004. p. 48-49.
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Ordering Information

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

Symbols used with IVD devices

