

Immunoglobulin M FS*

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

Order Information

Cat. No.	Kit size
1 7222 99 10 930	R1 4 x 20 mL + R2 2 x 8 mL
1 7222 99 10 935	R1 2 x 20 mL + R2 1 x 8 mL
5 9200 99 10 037	3 x 1 mL TruCal Protein high
5 9200 99 10 039	5 x 1 mL TruCal Protein: Calibrator set with 5 different levels

Summary [1-3]

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 immunoglobulin 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.

Method

Immunturbidimetric test

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

The reagents are stable up to the end of the indicated month of expiry, if stored at 2 – 8°C and contamination is avoided. Do not freeze the reagents and store them protected from light!

Warnings and Precautions

1. The reagents contain sodium azide (0.95 g/L) as preservative. Do not swallow! Avoid contact with skin and mucous membranes!
2. Reagent 2 contains animal material. Handle the product as potentially infectious according to universal precautions and good laboratory practice.
3. In very rare cases, samples of patients with gammopathy might give falsified results [8].
4. Please refer to the safety data sheets and take the necessary precautions for the use of laboratory reagents. For diagnostic purposes, the results should always be assessed with the patient's medical history, clinical examinations and other findings.
5. For professional use only!

Waste Management

Please refer to local legal requirements.

Reagent Preparation

The reagents are ready to use.

Materials required but not provided

NaCl solution 9 g/L
General laboratory equipment

Specimen

Serum, heparin plasma or EDTA plasma		
Stability [4]:	7 days at	20 – 25°C
	3 months at	4 – 8°C
	6 months at	-20°C

Only freeze once!

Discard contaminated specimens!

Assay Procedure for Analyzers

Application sheets for automated systems are available on request.

Wavelength	415/700 nm (bi-chromatic)
Optical path	1 cm
Temperature	37°C
Measurement	Against reagent blank

	Blank	Sample or calibrator
Sample or calibrator	-	2 µL
Dist. water	2 µL	-
Reagent 1	250 µL	250 µL
Mix, incubate for 3 min., read absorbance (A1), then add:		
Reagent 2	50 µL	50 µL
Mix, incubate for 3 min., read absorbance (A2).		

$$\Delta A = (A2 - A1) \text{ sample or calibrator}$$

Calculation

The IgM concentration in unknown samples is derived from a calibration curve using an appropriate mathematical model such as logit/log. The calibration curve is obtained with 5 calibrators at different levels and NaCl solution (9 g/L) for determination of the zero value.

Stability of calibration: 4 weeks

Conversion factor

$\text{IgM [mg/dL]} \times 0.0103 = \text{IgM [\mu mol/L]}$

Calibrators and Controls

For the calibration of automated photometric systems, DiaSys TruCal Protein calibrator set or TruCal Protein high calibrator are recommended. The assigned values of these calibrators have been made traceable to the reference material ERM[®]-DA470k/IFCC. DiaSys TruLab Protein control should be assayed for internal quality control. Each laboratory should establish corrective action in case of deviations in control recovery.

	Cat. No.	Kit size
TruLab Protein Level 1	5 9500 99 10 046	3 x 1 mL
TruLab Protein Level 2	5 9510 99 10 046	3 x 1 mL

Performance Characteristics

Measuring Range

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.

If results are below the lower range, repeat measurement with double sample volume. If results are still below the range, check for prozone effect by diluting the sample.

Prozone Limit

No prozone effect was observed up to an IgM value of 8000 mg/dL.

Specificity/Interferences

Due to its antibodies, DiaSys Immunoglobulin M FS 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. For further information on interfering substances refer to Young DS [5].

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 n = 40	Mean [mg/dL]	SD [mg/dL]	CV [%]
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 n = 40	Mean [mg/dL]	SD [mg/dL]	CV [%]
Sample 1	87.3	1.78	2.04
Sample 2	275	3.43	1.25
Sample 3	420	7.12	1.69

Method Comparison

A comparison of DiaSys Immunoglobulin M FS (y) with a nephelometric test (x) using 77 samples gave following results: $y = 0.93 x + 4.23 \text{ mg/dL}$; $r = 0.992$.

Reference Range

Adults [6]	40 – 230 mg/dL	0.41 – 2.37 $\mu\text{mol/L}$
Children [7]		
Newborns	10 – 30 mg/dL	0.10 – 0.31 $\mu\text{mol/L}$
1 – 3 month(s)	10 – 70 mg/dL	0.10 – 0.72 $\mu\text{mol/L}$
4 – 6 months	20 – 100 mg/dL	0.21 – 1.03 $\mu\text{mol/L}$
7 – 12 months	30 – 100 mg/dL	0.31 – 1.03 $\mu\text{mol/L}$
2 years	40 – 140 mg/dL	0.41 – 1.44 $\mu\text{mol/L}$
3 – 5 years	40 – 180 mg/dL	0.41 – 1.85 $\mu\text{mol/L}$
6 – 9 years	40 – 160 mg/dL	0.41 – 1.65 $\mu\text{mol/L}$
10 – 13 years	40 – 150 mg/dL	0.41 – 1.55 $\mu\text{mol/L}$

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

Literature

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Manufacturer



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