



IgM



Quantitative turbidimetric assay for the measurement of IgM in human serum or plasma



ORDER INFORMATION

REF	Kit size
GD8487 00	2x20 ml
KL8487 00	2x20 ml
BK8487 00	1x60 ml

CLINICAL SIGNIFICANCE

Quantification of immunoglobulins in serum is important for the diagnosis of primary or secondary immunodeficiency, for monitoring of immunoglobulin therapy, and for following the clinical course of multiple myeloma⁽⁴⁾.

IgM is the only immunoglobulin synthesized by neonates. In adult serum, represents the 5 to 10% of the total immunoglobulins. It is a pentameric molecule, and its big size prevents its passage the extravascular spaces.

Congenital and acquired immunodeficiency are causes of IgM deficit^(3,6).

Polyclonal hyperimmunoglobulinemia (normal response to infections) increases IgM concentration, specially in primary viral infections and blood stream infections such as malaria. IgM increases also are found in primary biliary cirrhosis and chronic active hepatitis.

Increments of monoclonal IgM (paraprotein) are found in proliferative disorders of plasma cells as Waldenström's macroglobulinemia⁽⁴⁾.

METHOD PRINCIPLE

IgM is a quantitative turbidimetric assay^(1,2) for the measurement of IgM in human serum or plasma.

Anti-human IgM antibodies form insoluble complexes when mixed with samples containing IgM. The scattering light of the immunocomplexes depends of the IgM concentration in the patient sample, and can be quantified by comparison from a calibrator of known IgM concentration.

COMPOSITION

Reagent A: Goat antibodies anti-human IgM, Tris buffer 20 mmol/l, pH 8.2. Sodium azide 0.95 g/l.

PREPARATION OF THE REAGENTS

Reagent A is ready to use.

Calibration curve

Dilute the Plasma Protein Calibrator in NaCl 9 g/l as follows:

Dilution	1	2	3	4	5	6
Calibrator (µl)	--	10	25	50	75	100
NaCl 9 g/l (µl)	100	90	75	50	25	--
Factor	0	0.1	0.25	0.5	0.75	1.0

Multiply the concentration of the calibrator by the corresponding factor to obtain the IgM concentration of each dilution.

STORAGE AND STABILITY

Reagent A is stable up to the date stated on the label, if contamination and evaporation are avoided.

The above conditions are valid if the vial is opened just only for the time to take the reagent, closed immediately with its cup and stored at the indicated conservation temperature.

Do not use the reagent after the expiry date.

Presence of particles, turbidity and/or the absorbance of blank reagent > 0.300 at 340 nm are sign of deterioration.

ANCILLARY EQUIPMENT

- Automatic pipette to measure reagent and sample
- Thermostatic bath at 37 °C

- Spectrophotometer or photometer thermostatable at 37 °C capable to read 340 ± 20 nm
- Analysis cuvettes (optical path = 1 cm)
- NaCl (9 g/l) solution
- Plasmaprotein Multicalibrator L (Ref. GD8469 00)
- Plasmaprotein Normal Control L (Ref. GD8461 00)
- Plasmaprotein Pathological Control L (Ref. GD8464 00)
- Plasmaprotein Normal and Pathological Control L (Ref. GD8466 00)

SAMPLES

Fresh serum and EDTA or heparinized plasma.

IgM in serum or plasma is stable 7 days at 2-8 °C or 3 months at -20 °C.

Samples with presence of fibrin should be centrifuged before testing. Highly hemolyzed or lipemic samples are not suitable for testing.

PROCEDURE

1. Prewarm the reagent and the photometer (cuvette holder) to 37 °C.
2. Using distilled water zero the instrument at 340 nm.
3. Pipette into a cuvette:

Sample / Calibrator	10 µl
Reagent (RA)	1000 µl

4. Mix well and insert the cuvette into the photometer. Record the absorbance (A) after 2 minutes of the sample or calibrator addition.

CALCULATION

Plot the different absorbance values (A) against the IgM concentration of each calibrator dilution. IgM concentration in the sample is calculated by interpolation of its (A) value in the calibration curve.

REFERENCE VALUES

Adults ⁽³⁾	40 – 230 mg/dl
Newborn ⁽⁴⁾	5 – 30 mg/dl

It is recommended that each laboratory establishes its own reference range according to the examined population.

QUALITY CONTROLS

To ensure adequate quality control (QC), each run should include a set of controls (normal and abnormal) with assayed values handled as unknowns.

Each laboratory should establish its own Quality Control scheme and corrective actions if controls do not meet the acceptable tolerances.

ANALYTICAL PERFORMANCE

Linearity

The method is linear up to 500 mg/dl, under the described assay conditions. Samples with higher concentrations should be diluted 1/5 in NaCl 9 g/l and retested again.

Detection limit

Values less than 3.0 mg/dl give non-reproducible results.

Analytical sensitivity

Using this reagent and method an ΔA of 1.2 mA at 340 nm is equivalent to 1 mg/dl of IgM at a concentration of 246 mg/dl.

Prozone effect

Prozone effect is not observed up to 1000 mg/dl of IgM.

Precision

mg/dl	Within-run		Between-run	
Mean		202.4	105.8	202.4
SD	4.2	7.7	6.5	12.2
%CV	3.9	3.8	6.1	6.0
N	10	10	10	10

Accuracy

Results obtained with this reagent did not show systematic differences when compared with commercial reagents of similar characteristics. Details of comparison are available on request.

Interferences

Bilirubin (20 mg/dl) and rheumatoid factors (400 UI/ml) do not interfere. Hemoglobin (4 g/l) and lipemia (1.25 g/l) may affect the results. Other substances may interfere⁽⁵⁾.

Note:

1. This method may be used with different instruments. Any application to an instrument should be validated to demonstrate that results meet the performance characteristics of the method. It is recommended to validate periodically the instrument. Contact to the distributor for any question on the application method.
2. The linearity limit depends on the sample/reagent ratio, as well as the analyzer used. It will be higher by decreasing the sample volume, although the sensitivity of the test will be proportionally decreased.
3. Clinical diagnosis should not be made on findings of a single test result, but should integrate both clinical and laboratory data.

PRECAUTIONS IN USE

The reagents contain inactive components such as preservatives (Sodium azide or others), surfactants etc. The total concentration of these components is lower than the limits reported by 67/548/ECC and 88/379/EEC directives about classification, packaging and labelling of dangerous substances. However, the reagents should be handled with caution, avoiding swallowing and contact with skin, eyes and mucous membranes.

The reagents from human donors have given negative results to anti-HIV 1/2, HBsAg and anti-HCV. It is recommended to handle with caution.

The use of the laboratory reagents according to good laboratory practice is recommended.⁽⁷⁾

Waste Management

Please refer to local legal requirement.

REFERENCES

1. Narayanan S. Clin Chem 128: 1528-1531 (1982).
2. Price CP et al. Ann Clin Biochem 20: 1-14 (1983).
3. Dati F et al. Eur J Clin Chem Clin Biochem 34 : 517-520 (1996).
4. Tietz Textbook of Clinical Chemistry, 3rd Ed. Burtis CA, Ashwood ER. WB Saunders Co., (1999).
5. Young DS. Effects of drugs on clinical laboratory tests. 3th ed. AACC Press (1997).
6. Friedman and Young. Effects of the disease on clinical laboratory tests, 3th ed. AACC Press, 1997.
7. EU-Dir 1999/11 Commission Directive of 8 March 1999 adapting to technical progress the principles of good laboratory practice as specified in Council Directive 87/18/EEC.