

PRINCIPLE:

The determination of human immunoglobulins is based on the reaction between immunoglobulin as antigen and the specific antiserum as antibody.

This reaction forms an insoluble complex producing a turbidity which is measured spectrophotometrically at 340 nm.

REAGENTS:

1. Reagent 1 (1x80 ml)

TRIS/PEG. buffer pH 7.5

2. Reagent 2 (1x2 ml)

Antiserum Anti-IgM

Optional: 101-0485 General proteins calibrator

PREPARATION AND STABILITY:

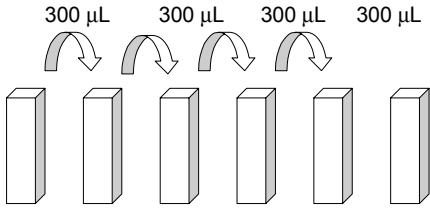
R.1: Ready to use. Stable at 2-8°C up to the date of expiration.

R.2: Must be diluted with buffer solution. The dilution depends on the analyser (Inquire).

Stable, at 2-8°C, up to the expiration date.

Calibrator; Ready to use.

Calibration curve: Prepare dilutions of the General Proteins calibrator using 9 g/L as diluent:

Std N°	1	2	3	4	5	6
Dilution	1/7	1/14	1/28	1/56	1/112	0
NaCl (µL)	600	300	300	300	300	300
Calibrator (µL)	100	--	--	--	--	--
						
Factor	3.0	1.50	0.75	0.38	0.19	0

Multiply the IgM calibrator concentration by the corresponding dilution factor indicated in the table to obtain the IgM concentration of the different calibrators.

SAMPLES:

Fresh serum.

Immunoglobulins in serum is stable 8 days at 2-8°C.

Do not use haemolized or lipemic samples.

The controls and samples will dilute manually or automatically with saline solution. (NaCl 0,9%).

PROCEDURE:

Wavelength: 340 nm
 Cuvette: 1 cm light path
 Temperature: 37 °C
 Zero: distilled water

1. Dilute Antiserum Anti-IgM (R.2) 1:41 with buffer solution R.1. The working reagent is stable 2 weeks at 2-8°C.

2. Dilute samples and controls 1:21 with saline solution. (NaCl 0.9%)

3. Pipette into a cuvette:

	Blank	Calibrator	Sample
NaCl 9 g/L (µL)	200	--	--
Calibrator (µL)	--	200	--
Dil. Sample (µL)	--	--	200
Work. Reag. (mL)	1.0	1.0	1.0

4. Mix and read the absorbance (A) against blank after 10 minutes of the working reagent addition.

CALCULATION:

Calculate the absorbance for each calibrator and plot the values found against the concentration in a calibration curve. IgM concentration in the sample is calculated by interpolation its A value on the calibration curve.

Chronolab has instructions sheets available for several automatic analyzers.

REFERENCE VALUES:

Between 60 – 280 mg/dL.

Each laboratory should establish its own reference range.

PERFORMANCE CHARACTERISTICS:

1. *Measurement interval:* 40 – 800 mg/dL, under the described assay conditions.