

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-IgA

Optional: 101-0485 General proteins calibrator

PREPARATION AND STABILITY:

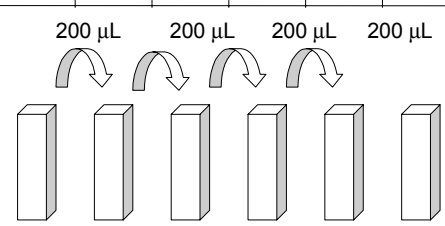
R.1: Ready to use. Stable at 2-8°C up to the date of expiration.

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 | 1/10 | 1/20 | 1/40 | 1/80 | 1/160 | 0 |
| NaCl (µL) | 450 | 200 | 200 | 200 | 200 | 200 |
| Calibrator (µL) | 50 | -- | -- | -- | -- | -- |
| |  | | | | | |
| Factor | 2.1 | 1.05 | 0.52 | 0.26 | 0.13 | 0 |

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

SAMPLES:

Fresh serum.

Immunoglobulins in serum are 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-IgA (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) | 50 | -- | -- |
| Calibrator (µL) | -- | 50 | -- |
| Dil. Sample (µL) | -- | -- | 50 |
| 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. IgA 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 90 – 450 mg/dL.

Each laboratory should establish its own reference range.

PERFORMANCE CHARACTERISTICS:

1. *Measurement interval:* 60 – 1400 mg/dL, under the described assay conditions.