# C3 Turbidimetric 5+1

Quantitative determination of C3 in human Serum and Plasma. Turbidimetric method.

**REF TB1093** R1: 2x25 mL + R2: 1x10 mL

## METHOD AND PRINCIPLE

Human Anti C3 (Antibody) is added to samples containing C3 (Antigen) and develops insoluble complexes. The solution increases its absorbance which is photometrically measurable at 340 nm. The amount of this increase in absorbance is directly proportional to the concentration of C3 present in the sample. The use of a calibration curve with known values makes it possible to determine the concentration of C3 in the unknown sample.

## CLINICAL SIGNIFICANCE

The complement is a set of proteins that are part of the Immune System that are activated as a result of bacterial infections, or as a result of inflammation or other diseases. The basic purpose of the complement is to protect the body by removing pathogens (bacteria, viruses, etc.) by facilitating their elimination or their control by other biological systems, serum or cellular.

C3 and C4 are used to determine whether an illness or a particular condition of a patient is caused entirely or in part by deficiencies or anomalies in the complement system. The C3 fraction is a complement protein that is activated when the body recognizes the presence of bacterial cells, or immune complexes. It can be activated in turn by the C4 fraction. Following the activation of the complement there is an immune response from the body. The complement measurement may be required to facilitate the diagnosis of the cause of recurrent microbial infections, angioedema or inflammation. It can also be used to aid in the diagnosis and to monitor the activity of acute and chronic autoimmune diseases, such as Systemic Lupus Erythematosus (SLE). It can be dosed and monitored in relation to diseases related to immune complexes such as: glomerulus nephritis (a renal disorder), serum sickness, rheumatoid arthritis and vasculitis (inflammation of blood vessels). When immunocomplexes are formed, the complement helps to eliminate them from the blood, consequently its blood levels are lowered.

## REAGENT COMPOSITION

**R1 (Buffer):**

| | |
|---|---|
| Tris Buffer, pH 7,5 | 100 mmol/L |
| Sodium Chloride | 150 mmol/L |

**R2 (Antiserum):**

| | |
|---|---|
| Tris Buffer, pH 7,5 | 100 mmol/L |
| Sodium Chloride | 150 mmol/L |
| Anti-human C3 Antibody (Goat) | |

## REAGENT PREPARATION AND STABILITY

Liquid and ready to use reagents, stable until the expiry date shown, if stored as indicated on the label and avoid contamination, evaporation and prolonged exposure to direct light. Do not freeze the reagents.

Discard the reagent if signs of deterioration appear, such as the presence of particles and turbidity or failure to recover the values of certified control sera or Absorbance of Blank Reagent >0.300 at 340 nm in cuvette 1 cm against water. After opening the bottles, it is advisable to withdraw the necessary volume, to immediately close the bottles and store them in the fridge in order to avoid contamination, degradation from direct light and evaporation.

Antibody Start (two-Reagents)

The Reagents are ready-to-use.

Sample Start (Mono-Reagent)

Mix 5 parts of **R1** + 1 part of **R2.** (e.g. 10 mL of R1 + 2 mL of R2) to obtain the Working Solution. Avoiding foaming, shake gently before to use.

Stability: 6 mounths at +2° to + 8°C; 5 days at +15° to + 25°C, 30 days on board (cooled rack).

## SPECIMEN

Serum, Li-Heparin or EDTA Plasma.

Avoid hemolysate or lipemic samples. Separate the serum from the clot quickly. Defrost only once. Stability in serum or plasma:

4 days at +2 to +8 °C, 1 day at 20-25° C; 3 months at -20°C.

## PROCEDURE

| | |
|---|---|
| Wavelength: | 340 nm |
| Temperature: | +37°C |
| Measurement: | against distilled water |

Antisera Start procedure:

| | |
|---|---|
| Reagent (R1) | 700 µL |
| Sample / Calibrator /$H_2O$ | 12 µL |

Mix and after 30" read Absorbance (Abs1). Then add:

| | |
|---|---|
| Reagent (R2) | 140 µL |

Mix, after other 300" read Absorbance again (Abs 2).

Calculate ΔAbs (Abs 2 – Abs 1) for samples and calibrators.

Sample Start procedure**:**

| | |
|---|---|
| Working Solution | 700 µL |
| Sample / Calibrator/$H_2O$ | 10 µL |

Mix, read Absorbance (Abs 1) after 30".

After other 300" read Absorbance again (Abs 2).

Calculate ΔAbs (Abs 2 – Abs 1) for samples and calibrators.

## CALIBRATION

The results depend on the accuracy of the calibration, on the correct setting of the test on the instrument, on the correct volumetric ratio of reagent / sample and on the correct analysis temperature.

Use **MTD Diagnostics** Plasma Protein Multicalibrator

**Plasma Protein Multicalibrator REF TUC1030 (3x1 mL)**

Dilute calibrator in NaCl 9g/L as follows:

| **Dilution** | 1 | 2 | 3 | 4 | 5 | 6 |
|---|---|---|---|---|---|---|
| **CAL (µL)** | -- | 10 | 25 | 50 | 75 | 100 |
| **NaCl 9 g/L (µL)** | 100 | 90 | 75 | 50 | 25 | -- |
| **Factor** | 0.0 | 0.1 | 0.25 | 0.5 | 0.75 | 1.0 |

Multiply the concentration of Protein Calibrator by the corresponding factor to obtain the concentration of each dilution.

To avoid to dilute the Multicalibrator, it is possible to use:

**Plasma Protein Multicalibrator Set REF TUC1035 (5x1 mL)**

a multipoint calibration curve in pre-filled vials, each with a specific concentration. The values are shown on the label of each vial.

## CALCULATION

The analytical session can not be validated if the Δ Abs (Abs2 - Abs1) of the Blank Reagent is > 0.300 in a 1 cm cuvette of optical path.
Plot the different ΔAbs (Abs2-Abs1) absorbances against the concentration of each calibrator dilution. The concentration of the sample is calculated by interpolation of its ΔAbs (Abs2-Abs1) value on the calibration curve. For automatic calculation, use the SPLINE curve but other mathematical method can be used (Point-Point; Logit-Log 4P, etc..).

Conversion Factor: mg/dL x 0,01 = g/L ; mg/L x 0,1 = mg/dL

## QUALITY CONTROL

Normal and abnormal control sera of known concentration should be analysed routinely with each group of unknown samples utilizing **MTD Diagnostics** Quality Control Material:

**Plasma Protein Control Level 1 REF: TUC1040 (3 x 1 mL)**
**Plasma Protein Control Level 2 REF: TUC1050 (3 x 1 mL)**

The range of the values of the controls must be evaluated as a guideline, since it can be determined by the application of the method or by the user's manual skills or by other factors. The values obtained must be used for the evaluation of the Precision of the method (Repeatability). For the evaluation of the Accuracy of the method (Reproducibility) it is necessary to adhere to a program of External Quality Assessment (EQA) managed by certified bodies.

## EXPECTED VALUES

Adults: 90 - 180 mg/dL

Each laboratory should establish a range of expected values based on its patient population and, if necessary, determine its own reference interval. For diagnostic purposes, results should always be assessed together with the patient's medical history, clinical examination and other results.

## PERFORMANCE

PRECISION:
Low Level: Samples (n) = 20; Average = 125.8; S.D. = 2.53; CV = 2.01%
High Level: Samples (n) = 20; Average = 179; S.D. = 2.32; CV = 1.29%

ACCURACY (CORRELATION): A comparison between this method (x) and a certified method of trade (y) gave the following correlation:

**y = 1.002 x – 0.71 r = 0.999**

SENSITIVITY: 4 mg/dL.

LINEARITY: 4 – 400 mg/dL.

## SPECIFICITY / INTERFERENCES

No interferences was observed by Bilirubin up to 20 mg/dL, Hemoglobin up to 1000 mg/dL, Triglycerides up to 800 mg/dL.
Other substances may interfere.

## PRECAUTIONS

R1 and R2 contain TRIS BUFFER 100 mmol/L – pH 7.5 - CAS 1185-53-1

H315: Causes skin irritation
H319: Causes serious eye damage
H335: May cause respiratory irritation

The antibody present in the preparation are of animal origin and are not capable of transmitting infectious diseases to humans. However, since there are no methods to ensure the total absence of such infectious agents or of other microbes, this product must be handled as if it were risky and potentially capable of transmitting infectious diseases of any kind, in accordance with Good Laboratory Practice standards.
The products do not contain other dangerous substances or mixtures, according to the EC Regulation n ° 1272/2008 or their concentrations are such as not to be considered persistent, bioaccumulative or toxic (PBT). The product is classified and labeled in accordance with EC directives or respective national laws. Sodium azide, less than 0.1%.
However, in compliance with the normal prudential rules that everyone has to keep managing any chemical or laboratory reagent, in case of contact of reagents with the operator, you must apply the following first aid:
S26 (P305 – P351 – P338): In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.
S28 (P302 – P352): After contact with skin, wash immediately with plenty of water.
S36/37/39 (P280): Wear suitable protective clothing, gloves and eye/face protection.
S46 (P301 – P310): If swallowed, seek medical advice immediately and show container or label. If victim is conscious and alert, wash out mouth with water.
S56 (P273): Dispose of this material and its container at hazardous or special waste collection point.
S63 (P304 – P340): In case of accident by inhalation: remove casualty to fresh air and keep at rest. If breathing is difficult, give oxygen and get medical aid.
All samples, calibrators and controls should be treated as potentially infectious material capable of transmitting HIV and hepatitis.
FOR MORE INFORMATION, REQUEST SAFETY DATA SHEET FOR REAGENT (MSDS) AT THE MANUFACTURER.

## SIMBOLOGY

| | | | |
|---|---|---|---|
| CE | CE Mark (EC Directive 98/79) | | |
| IVD | In Vitro Diagnostic |  | Temperature Limitation |
|  | Consult instructions for use |  | Contains sufficient for <n> test |
| REF | Catalog Number |  | Use By |
| LOT | Batch Code |  | Manufacturer |

## BIBLIOGRAPHY


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