

NEO TURBI

COMPLEMENT - C4

(Turbidimetry Method)

| KIT NAME | KIT SIZE |
|----------------|-----------|
| NEO TURBI - C4 | 1 x 40 ml |

INTRODUCTION

Complement C4 (C4) is intended for Invitro quantitative determination of C4 in human serum. Complement component 4 (C4) is a protein of the complement system. It is activated in the classical pathway which leads to the formation of the C3 convertase. C4 plays vital role in tissue cell damage and inflammation in immunologic disorders. Decreased levels of C4 may be the result of systemic lupus erythematosus (SLE), hereditary angioedema, autoimmune hemolytic anemia, and autoimmune nephritides. Conversely, C4 levels increases in a variety of inflammatory and necrotic disorders as part of the acute-phase plasma protein response.

METHOD PRINCIPLE

The Kit utilizes latex-enhanced immunoturbidimetry to measure the C4 level in human serum or plasma. During the test, C4 in the sample binds with the specific anti C4 antibody to cause agglutination. The turbidity caused by agglutination is detected optically by chemistry, analyzer. The change in absorbance is proportional to the level of C4 in the sample. The actual concentration is obtained by comparing with a calibration curve with known concentrations

KIT CONTENTS

| | |
|--------------------|-----------|
| R1 - C4 Buffer | 1 x 30 ml |
| R2 - C4 antibody | 1 x 10 ml |
| R3 - C4 Calibrator | 0.5 ml |

The reagents when stored at 2-8°C are stable up to expiry date printed on the package. The reagents are stable for 7-10 days on board the analyser at 2-10°C. Protect from light and avoid contamination.

WORKING REAGENT PREPARATION AND STABILITY

Assay can be performed with use of separate R1-C4 and R2-C4 reagents of 3 parts of R1-C4 with 1 part of R2-C4. Avoid foaming.

CONCENTRATIONS IN THE TEST

R1 - Phosphate buffer, Polyethylene glycol, Sodium azide < 0.1%
R2 - Anti-C4 antibodies, Tris buffer, sodium azide < 0.1%

WARNINGS AND NOTES

1. The Kit is for in vitro diagnostic use only. Not for use in humans or animals.
2. The instructions must be followed to obtain accurate results.
3. Do not use the reagents beyond the expiration date.
4. Treat all specimens as infectious. Proper handling and disposal procedures of specimens and test materials should be strictly followed

ADDITIONAL EQUIPMENT

- Automatic analyzer or photometer able to read at 340 nm
- Thermostat at 37°C
- General laboratory equipment

SPECIMEN

Follow standard laboratory procedures to collect serum samples. It is recommended to perform test immediately after sample collection. If the test cannot be done immediately, store sample at 2-4° C for up to 3 days or at -20° C for up to 1 months. Avoid repeated freezing and thawing.

PLOTTING OF MULTIPOINT CURVE

The Turbichem C4 is based on Non-Linear Reactions, hence it is strongly recommended to run Multi-standard mode to plot the Multi-point curve to have better accuracy and precise result.

Serial Dilution Step

| | 1st | 2nd | 3rd | 4th | 5th |
|-------------------|--------|---------------------|---------------------|---------------------|---------------------|
| Calibrator | 100 µl | 50 µl from 1st Tube | 50 µl from 2nd Tube | 50 µl from 3rd Tube | 50 µl from 4th Tube |
| Normal Saline | 0 | 50 µl | 50 µl | 50 µl | 50 µl |
| Ratio of Dilution | Neat | 1/2 | 1/4 | 1/8 | 1/16 |

PROCEDURE

These reagents may be used both for manual assay and in several automatic analyzers. Programme Sheets are available on request.

Wavelength 340 nm
Temperature 37°C
Cuvette 1 cm

Pipette into the cuvette:

| Reagent | Blank | Calibrator (C) | Test (T) |
|---|--------|----------------|----------|
| R1 C4 Buffer | 750 µl | 750 µl | 750 µl |
| Calibrator | - | 10 µl | - |
| Sample | - | - | 10 µl |
| Distilled Water | 10 µl | - | - |
| Mix well and incubator for 5 mins. at 37° C | | | |
| R2 C4 Antibody | 250 µl | 250 µl | 250 µl |

Mix well & incubate for 5 min. at 37°C. Measure the absorbance of calibrator & sample against reagent blank (B).

CALCULATION

C4 concentration = $\frac{\text{Abs. Test}}{\text{Abs. Calibrator}} \times \text{Calibrator Concentration}$

REFERENCE VALUES

17 to 48 mg/dL

It is recommended for each laboratory to establish its own reference ranges for local population.

QUALITY CONTROL

To ensure adequate quality control, each run should include assayed normal and abnormal controls. If commercial controls are not available it is recommended that known value samples be aliquoted, frozen and used as control.

PERFORMANCE CHARACTERISTICS

- **Linearity** : 0 to 85 mg/dL
- **Precision** : within Run CV ≤ 6 %
- **Specificity / Interferences**

No interference detected for bilirubin upto 60 mg/dL and hemoglobin 10 g/L, triglycerides 1000 mg/dL.

WASTE MANAGEMENT

Please refer to local legal requirements.

LITERATURE

1. Burtis C, Ashwood, ER (ed). Tietz Textbook of Clinical Chemistry, 3rd ed. Philadelphia, PA; WB Saunders Co; 506; 1999.

2. Liu CC, Manzi S, Kao AH, Navratil JS, Ruffing MJ, Ahearn JM (2005). "Reticulocytes bearing C4d as biomarkers of disease activity for systemic lupus erythematosus". Arthritis Rheum. 52 (10): 3087-99.

3. Wouters D, Wiessenberg HD, Hart M, et al. Complexes between C1q and C3 or C4; novel and specific markers for classical complement pathway activation. J Immunol Methods, 2005, 298(1-2); 35-45.

SYSTEM PARAMETERS

| | |
|--------------------------|-----------------------|
| Method | End Point |
| Wavelength | 340 nm |
| Zero Setting Temperature | Reagent Blank |
| Setting Incubation | 37° C |
| Temperature Incubation | 37° C |
| Time | 5 mins. + 5 mins |
| Delay Time | ---- |
| Read Time | ---- |
| No. of Reading | 2 |
| Interval Time | ---- |
| Sample Volume | 0.01 ml (10 ul) |
| Reagent Volume | 1.0 ml (1000 ul) |
| Calibrator Concentration | Refer Calibrator vial |
| Units | mg/dl |
| Factor | ---- |
| Reaction Slope | Increasing |
| Linearity | 85 mg/dl |