

# TURBICHEM COMPLEMENT - C4

(Turbidimetry Method)

KIT NAME	KIT SIZE	CAT. NO
Turbichem - C4	1 x 40 ml	TCC400040D



## 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	1 vial

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. Applications for them are available on request.

Wavelength 340 nm  
Temperature 37°C  
Cuvette 1 cm

## Pipette into the cuvette:

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

Mix well & incubate for 5 min. at 37°C. Measure the absorbance of calibrator & sample

## 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 64.0 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	Reagent Blank
Temperature Setting	37° C
Incubation Temperature	37° C
Incubation 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)
Standard Concentration	Refer Calibrator vial
Units	mg/dl
Factor	----
Reaction Slope	Increasing
Linearity	64 mg/dl



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