TurbiMAX C4 Factor

Turbidimetric Immuno Assay (TIA)

ORDERING INFORMATION:

Ref./Cat.	Pack Size	Presentation
AVC4T-24	24 ml	Two Reagents with 4 level Calibrators

PRODUCT FEATURES

- Turbidimetric Immuno Assay (TIA)
- Liquid Stable Two Reagents
- 4 Level Lyophilized Calibrators Provided
- 5 Minutes End Point Bichromatic Reaction
- Measurement at 340 nms
- Test Procedure time 5 minutes at 37°C
- Linearity : 100.0 mg/dL
- Adaptable to Semi and Automated Analyzers

INTENDED USE:

Kit is use for the Quantitative Determination of C4 Factor in Human Serum

CLINICAL SIGNIFICANCE:

All complement proteins are acute phase reactants and rise rapidly in concentrations during inflammatory episodes. Conversely, the rates of complement protein catabolism may greatly increase in various autoimmune diseases. Because complement component determinations represent a static measurement of the net concentrations that result from a dynamic balance between component synthesis and catabolism, serial sample quantitations are more clinically useful. In most disease states, complement functions “normally” in producing inflammation and tissue damage. When complement plays a role in the

development of a disease, it is often due to activation by an “abnormal” antibody, immune complex, or foreign material.

Increased C4 levels are associated with acute phase reactions and certain malignancies.

Decreased levels of C4 occur in individuals with congenital deficiency or immunologic diseases (where complement is consumed at an increased rate). C4 levels may be decreased in hereditary and acquired angioedema, complement activation due to immune complex diseases, decreased synthesis due to liver disease, increased consumption in glomerulonephritis, systemic lupus erythematosus (SLE), rheumatoid arthritis, respiratory distress syndrome, autoimmune hemolytic anemia, cryoglobulinemia, and sepsis.

Total congenital C4 deficiency is rare, but partial C4 deficiency is common. Partial and complete congenital C4 deficiencies have been associated with immune complex diseases, SLE, autoimmune thyroiditis, and juvenile dermatomyositis. Infections associated with C4 deficiency include bacterial or viral meningitis, Streptococcus and Staphylococcus sepsis, and pneumonia.3Refer to the following table for a general guide to evaluation of Complement C3 (C3) and C4 protein levels in the presence of decreased hemolytic complement activity

PRINCIPLE:

Quantitative determination of C4 may be done by an immunoturbidimetric method by automatic analyzers or in manual.Mixing a sample with a precise Antigen to a solution having the corresponding anti-serum (Antibody), in a well-defined ratio, it is possible to have turbidity; the use of undiluted sample may require bichromatism.

STORAGE AND STABILITY

All the reagents are stable up to the expiry date mentioned on the labels when the proper storage conditions are maintained.

KIT COMPONENTS

1. C4 Reagent R1
2. C4 Reagent R2
3. C4 Calibrators (4) : Concentration as stated on the label

COMPOSITION

Reagent 1:

(R1) 4-Hydroxyethyl Piperazine Ethanesulfonic acid 50mmol/L

Reagent 2:

(R2) Goat anti-human complement 4 antibody

REAGENT RECONSTITUTION & STABILITY

Reagent are liquid stable no need for reconstitution.

When the reagent is stored properly at 2-8°C & the contamination avoided, it is stable up to the expiry date mention on the label & kit box.

C4 Calibrators :

TurbiMAX Complement C4 is provided with 4 Levels of Lyophilized Calibrators. **Reconstitute each level with 0.5 ml of Distilled Water and keep it for 20 Minutes.** Mix gently and make a uniform suspension. Reconstituted Calibrators are stable for 60 Days once stored properly at 2-8°C. Aliquot it in to small volumes and store at 2-8°C for the contamination free use and for good reconstitution stability. Calibrators are stable for 6 Months when frozen at -20°C if the repeated freeze and thaw cycles are avoided.

Complement C4 Calibrators are validated with a traceability. Calibrators are calibrated to the Reference Material CRM 470/RPPHS (Institute for Reference Materials and Measurements).

MATERIAL REQUIRED BUT NOT PROVIDED

Laboratory Instrumentation, Spectrophotometer UV/VIS with thermostatic cuvette holder or clinical chemistry analyzer: semi auto, calibrated micropipettes, glass or high quality polystyrene cuvettes, test tube/rack, heating bath controls, saline.

REAGENT DETERIORATION

Discard any turbid reagent if blank reagent absorbance exceeds 0.5 at 340 nm against distilled water.

WARNING & PRECAUTIONS

- Reagent may contain some non reactive and preservative components. It is recommended to handle carefully, avoiding contact with skin and ingestion.
- Specimen should be considered infectious and handled appropriately.
- Contamination by soap or glycerol will affect this assay.
- Perform the test according to the general “ Good Laboratory Practice” GLP

SPECIMEN COLLECTION & STORAGE

Not haemolysed and non lipemic fresh serum.
Samples collection in compliance with CLSI (NCCLS)
The sample can be stored at 2-8°C up to 6 days.

SYSTEM PARAMETERS:

Reaction type : **End Point-Bichromatic- Non Linear- Multi Cal- Spline**

Reaction Direction	: Increasing
Primary Wave Length	: 340 nm
Secondary Wave Length	: 700 (600-700) nm
Flow cell Temp.	: 37°C
Sample volume	: 5 µl
Reagent volume	: R1 350 µl + R2 70 µl
Calibrators Conc: 1,2,3,4	: Lot Specific (Check the labels)
Units	: mg/dL
Blanking with	: Reagent
Low normal	: 15
High normal	: 53 Male, 57 Female
Linearity	: 100

TEST PROCEDURE

Reagent	Reagent Blank	C	S
Reagent R1	350 µL	350 µL	350 µL
Calibrators (1,2,3,4)	----	5 µL	----
Sample	----	----	5 µL
Incubate 5 Minutes at 37°C			
Reagent R2	70 µL	70 µL	70 µL

Mix carefully and incubate at 37°C for 5 minutes, Measure the absorbance of calibrators and of the samples against reagent blank.

CALCULATIONS:

The Multipoint Non Linear /Semi logarithmic calibration model was used , and the Spline function was used as the calculation model. The dose / response curve was made based on the value of the calibrator and the change of absorbance. The concentration of C4 in the sample could be calculated on the dose/ response curve based on the change of absorbances

EXPECTED VALUES:

1-14 Years	Male:	14 - 44
	Female:	13 - 46
Above 14 - 80 Years	Male:	15 - 53
	Female:	15 - 57

QUALITY CONTROL & CALIBRATION:

Quality Control sera are recommended to monitor the performance of manual and automated assay procedures. TurbiMAX C4 Specific Proteins Controls are available optionally

Each laboratory should establish its own Quality Control scheme and corrective actions if controls do not meet the acceptable tolerances.

PERFORMANCE CHARACTERESTICS:

1. Linearity

Linearity : 100 mg/dL.

2. Sensitivity/ Limit of Detection (LOD)

The lower limit of detection is 1.5 mg/dL.

3.Interferences:

Interference test criterion: Recovery ± 30% of initial value. No interference found on samples with: Total bilirubin up to 20 mg/dL; Haemoglobin up to 150 mg/dL; Acorbic acid up to 50 mg/dL.

4. Precision: The reagent has been tested for 20 days, using two levels of serum in a EP5-based study (NCCLS).
Determined on 20 replications of 2 samples.
The results obtained are following:

Intra-Assay

N=20	Mean (mg/dl)	SD (mg/dl)	CV%
Control serum 1	16.5	0.5	3.03
Control serum 2	74.6	0.6	0.80

Inter-Assay

N=20	Mean (mg/dl)	SD (mg/dl)	CV%
Control serum 1	16.8	0.65	3.86
Control serum 2	75.1	0.73	0.97

5. Method Comparison:

Results obtained using this reagent (y) were compared to those obtained with a Bayer immunoturbidimetric method. 39 samples ranging from 50 to 100 mg/dL of C4 Factor were assayed.

Linear regression equation y = 1.0341 x - 14

Correlation coefficient r = 0.9721 n = 20

LIMITATIONS

Measuring range: 1.5-100 mg/dl. Determine samples having higher concentrations manually dilute with 0.9% NaCl or distilled/deionized water (e.g. 1 + 1). Multiply the result by the appropriate dilution factor (e.g. 2).

WASTE DISPOSAL

Reagents must be disposed off in accordance with local regulations.

NOTES

A) Applications on routine analyzers may be totally different from what developed as manual determination; in addition the procedures are specific for each analyzer.

B) Very deep attention must be given to interfering substances: certain drugs and other substances are able to influence levels of C4

C) The calibration curve has to be always repeated at each change of the lot of the Reagent and/or calibrator.

D) A proportional variation of the reaction volumes does not change the result.

E). For concentration of C4 higher than the maximum value of the Calibrator dilute the sample 1:5 with saline solution repeat the determination and multiply the result by 5.

REFERENCE

1. Clinical Guide to Laboratory Tests, Edited by NW Tietz W B Saunders Co., Phipladelphia, 483, 1983.
2. Yang Y et al. Curr Dir Autoimmun 2004: 7: 98-132.
3. Borque L et al. Clin Biochem 1983; 16: 330-333.
4. Pesce AJ and Kaplan, LA. Methods in Clinical Chemistry. The CV Mosby Company, St. Louis MO, 1987.
5. Dati F et al. Eur J Clin Chem Clin Biochem 1996; 34: 517-520.

Symbols Used on Pack

REF	Catalogue Number		Warning/Caution
LOT	Batch No.	IVD	In vitro diagnostic device
	Manufacturing Date		Storage Limit
	Expiry Date		Consult instruction for use
	Manufacturer		Keep away from sunlight
	Keep Dry		Do not use if package is damaged
	Contains sufficient no. of test		

ISO 13485 Certified Company

5 Schedule MDR 2017

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