Pariksha Biotech
A game changer in IVD

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Complement C4 Estimation Kit

High-Q C4 Factor

Turbidimetric Immuno Assay (TIA)

Intended 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

	Normal C4	Decreased C4
Normal C3	1) Alterations in vitro (e.g., improper specimen handling) 2) Coagulation-associated complement consumption 3) Inborn errors (other than C3 or C4)	1) Immune Complex Disease 2) Hypergammaglobulinemic states 3) Cryoglobulinemia 4) Hereditary angioedema 5) Inborn C4 deficiency
Decreased C3	1) Acute glomerulonephritis 2) Membranoproliferative glomerulonephritis 3) Active SLE 4) Inborn C3 deficiency	1) Active SLE 2)Serum sickness 3) Autoimmune/chronic active hepatitis 4) Infective endocarditis 5) Immune complex disease

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.

Using our calibrator which is traceable to the CRM 470 International Standard it is possible to prepare a Calibration Curve to refer generally not rectilinear and not crossing the origin. Plotting Calibration Curve with the absorbance values and concentration of each calibrator it is possible to determine the concentration of human serum sample

Kit Components:

Reagent 1:

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

Reagent 2:

(R2) Goat anti-human complement C4 antibody

C4 Calibrators :

High-Q 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).

Stability

The Reagents are stable up to the expiry date mentioned on the labels when properly stored at 2-8°C.

Preparation of the reagent:

Reagents are supplied as ready to use reagents and do not requires reconstitution and working reagent preparation.

Samples

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.

Quality Control:

Quality Control sera are recommended to monitor the performance of manual and automated assay procedures. High-Q 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.

Test Procedure:

System Parameters:

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

Reaction Direction	: Increasing
Primary Wave Length	: 340
Secondary Wave Length	: 700 (600-700)
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

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

Mix carefully and wait for about 5 minutes. Measure the absorbance of calibrators and of the samples against blank 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

Within-run Precision: Determined on20 replications of 2 samples.
The results obtained are following:
Run-to-run Precision: Determined for 5 days with 20 replications for each days, for two samples.
The results obtained are the following:
Accuracy: A group of 20 sera has been tested using this procedure and using a similar reagent available on the market. The comparison gave these results:

Linear regression equation y = 1.0341 x - 14
Correlation coefficient r = 0.9721 n = 20

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

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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.

Attention:

The kit is tested for a manual spectrophotometer and for HITACHI, COBAS and MINDRAY systems. The applications on automatic analyzers could be completely different by what has been developed as manual determination.

Reference Values:

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

Reference ranges are based on a 95% confidence interval for a large Indian population. Confirmation of the reference ranges for individuals between the ages of 14 and 80 years was conducted using serum samples from 25 males and 52 females, based on Clinical and Laboratory Standards Institute (CLSI) protocol NCCLS C28-A2. It is recommended that each laboratory determine its own reference range based upon its particular locale and population characteristics.

Analytical Performances:

(Validate on MINDRAY Bs300)

The performances of the Reagent C4 FACTOR have been tested with a MINDRAY BS300 analyzer. The data, while representing the characteristics of the product, could be different for each laboratory and for different analyzers.

Sample	Mean (mg/dL)	2s	CV %
Human 1	13.8	1.5	5.6
Human 2	28.7	0.6	1.1

Sample	Mean (mg/dL)	2s	CV %
Human 1	13.5	1.0	3.5
Human 2	28.7	0.8	1.4

Method Linearity:

The test is linear up to 100.0 mg/dL.

However, for C4 concentrations higher than the maximum value of the calibrator, it is recommended to dilute the sample 1:5with saline solution, test again and multiply the result x 5.

Method Sensitivity (LoD): the sensitivity limit, that is the minimum concentration that can be distinguished by zero is 1.5 mg/dL.

BIBLIOGRAPHY:

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.

Ordering Information:

Ref./Cat.	Pack Size	Presentation
P-C4-24	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**

Symbols used with IVD devices

	Date of manufacture		Manufactured by
IVD	In vitro diagnostic device		Keep away from sunlight
	Do not freeze		This way up
	Use by (yyyy-mm-dd or mm/yyyy)	R	Reagent
CAL	Calibrator Material	LOT	Batch code
2°C 8°C	Temperature limitation (store at)	CONTROL	Control
	Consult instructions for use		Keep dry Keep away from rain
REF	Catalog Number		

eIFU Indicator

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SCAN TO EXPLORE MORE

Manufactured in India by :
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AN ISO 13485 Certified Company

Rev # 2