Complement C4 (C4)
(Immunoturbidimetric Method)

- **Kit Specifications:**

	Reagent/Quantity	Storage
Cat. No.: **CF0011**	R.1: 1 x 50 ml R.2: 1 x 10 total 60 ml	2-8°C
Cat. No.: **CF0017**	R.1: 1 x 50 ml R.2: 1 x 10 total 60 ml	2-8°C

- **Intended Use:**

In Vitro Diagnostic reagent pack for the quantitative determination of complement C4 in human serum/plasma on automated and semi-automated photometric systems.

- **Summary and Explanation:**

The function of C4 complement is a vital in immunity system. This complement in human serum contains 31 ingredients which act such as enzyme, cofactor, receptors, suppressor and protein to attached to the membrane. All the ingredients of this complement finally make cascade reactions to synthesis the proteins which facilitators to response of inflammation and immunity. The measurement of these complements are beneficial to diagnostic of genetic or adventitious disorders. Also, it is valuable for monitoring the activity of infection diseases and auto immune diseases. The decrease of the ingredients of this complement may be genetic or adventitious. Whenever the complement is active all components are consumed so the level of them derives down in serum. This cascade reaction in complement basically is in two ways classical and alternative and C4 is exist in both ways so whenever the concentration of C4 reduction which means they are consumed and high activity. C4 is a glycol protein contain three chains by disulfide binding with each other and 206 kilo Dalton. The Alfa chain have an ability to attached to the membrane. The beta and gamma chains both have an active site. This complement mainly produces in liver but macrophages and kidney tubules can be synthesis as well. However, the one part of C4 is C4b which has the ability to bond to the membrane as well as C2 partner make a C3 convertase. The C4 is only participate also active in classic way whenever the amount of it is normal and C3 is decrease so which means the alternative way being active. Reduction of the C4 can be observed in some complex immunity disease, systemic erythromatosis, auto immune thyroiditis and Juvenile dermatomyositis. The sole decline of this complement could be observe in genetic or adventitious angioneurotic edema disorders. However, in this genetic disorder the C3 and C4 decrease is reported. Therefore, the measurement and evaluation of C4 is useful also the increase of it is observed in pregnancy. Hence, C4 is a critical protein phase so the concentration will be increased in all inflammation reactions but if this amplification is twice than normal range so may fall or hidden the original amount.

Principle of the Method:

This method is based on reaction between anti-gen and anti-body. The C٤ exist on the sample become sensitive by anti-body against human C٤ and finally make turbid complex. The intensity of turbidity is directly related to amount of C۴ in the sample which measuring at 340 nm.

C4 antigen + Anti-C4 antibodies → Antigen/Antibody Complex

- **Reagent Preparation and Stability:**

Reagent is ready for use.
Before use, mix reagent by gently inverting each bottle.
Reagent is stable until the expiration date on the label when stored tightly closed at 2-8°C, protected from light and contaminations prevented during their use.
Do not use reagents over the expiration date.
Do not freeze and protect from light.
R1 is transparent and colorless.
R2 light cream color.
The following table is the preparation of calibrator with normal saline.
The normal saline 0.9%NaCl use as a zero.

Dilution	Neat	1:2	1:4	1:8	1:16
Dilution Factor	1	0.5	0.25	0.125	0.063

Waste Management:

Refer to local legal requirements for chemical disposal regulations.
Warning: Handle waste as potentially biohazardous material.
Dispose of waste according to accepted laboratory instructions and procedures.

- **Warnings and Precautions:**

For In Vitro Diagnostics Use Only.
For Professional Use Only.
Carefully read instructions for use.
In case of serious damage to the bottle or cap, resulting in product leakage or contamination, do not use the reagent pack and contact your distributor.
Take all necessary precautions required when handling laboratory reagents.
Do not use components past the expiry date stated on the Bottles.
Do not interchange caps among components as contamination may occur and compromise test results. For diagnostic purposes, the results should always be assessed with the patient's medical history, clinical examinations and other findings.
Any serious incident related to the product must be reported to the manufacturer and the competent authority of the Member State where the user and/or patient is located.

- **Type of Specimen:**

Use fresh serum, non-hemolysis, plasma collected by EDTA. The stability of C4 serum/plasma samples at 2-8°C in 7 days. The samples could be freeze for 7 days. Avoid any contamination.

Required but not Supplied:

Specific Protein calibrator from TKS or other valid calibrators.
Specific Protein Control Level 1 & 2 from TKS or other valid controls.
Saline solution 0.9 % NaCl
General laboratory equipments

- **Notes:**

Carefully read instructions for use.
It is recommended to use disposable material. If glassware is used the material should be scrupulously cleaned with hydrochloric acid 1 N and then thoroughly rinsed it with distilled water.
Use clean disposable pipette tips for its dispensation.
Disposable and glassware material used must be free of metals, ions and detergents.

Performance Characteristics:

Performance results can vary with the instrument used.
Data obtained in each individual laboratory may differ from these values.

The Maximum concentration will be obeying base of calibrator

LOD: 0.3 mg/dL

For samples with a higher concentration (base on maximum concentration of your calibrator), dilute 1:1 with 0.9 % NaCl and re-assay. Multiply result by 2.

Prozone:

In this assay till ۳00mg/dl concentration no prozone will not be observed.

Precision:

Intra Assay-Within run C4

Sample	n	Mean (mg/dl)	SD (mg/dl)	CV (%)
1	20	17	0.43	2.59
2	20	27	0.48	1.74

Inter Assay-Between run C4

Sample	n	Mean (mg/dl)	SD (mg/dl)	CV (%)
1	20	17	0.54	3.19
2	20	27	1.14	4.29

- **Accuracy:**

Results obtained using BIOMEDIC reagents (y) did not show systematic differences when compared with other commercial reagents (x).
Correlation coefficient (r): 0.999
Regression equation: Y = 1.001 (X) - 0.017 mg/dl
The results of the performance characteristics depend on the analyzer used.

- **Interfering Substances:**

Bilirubin (mixed isomer)	Less than 10% interference up to 600 µmol/L Bilirubin
Lipaemia	Less than 10% interference up to 5 g/L intralipid
Haemolysis	Less than 10% interference up to 5 g/L Hemoglobin.

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- **Reference Values:**

Units	mg/dl	mg/L
Adults	10-40	100-400

Each laboratory should establish its own expected values. The C4 results should always be reviewed with the patient's medical examination and history.

- **Assay Procedure:**

Allow reagents to reach working temperature before using.
A proportional variation of the reaction volumes indicated does not change the result

Assay conditions:

340 nm	**Wavelengths**
37 °C	**Incubation Temperature**
1 cm	**Cuvette**

Adjust the instrument to zero with distilled water.

Control/Sample/Calibrator	Blank	
1000 µl	1000 µl	**R1**
20 µl	-	**Control/Sample/Calibrator**
Gently mix and incubate for 5 minutes at 37°C. the first absorbance OD1 of sample measurement. Then added R2		

Control/Sample/Calibrator	Blank	
200 µl	200 µl	**R2**
Gently mix and incubate for 10 minutes at 37°C. The second absorbance OD2 of sample measurement.		

- **Calculations:**

Δ Abs= OD2 – OD1

The changes of absorbance Δ Abs should be followed by first absorbance and second absorbance respectively. Formerly, the second one should minus to the first one. Then the changes for all different calibrators should put in the logarithmic table so by this principal, the concentration of control and samples should be determining.

Conversion units:

mg/L = mg/dl x 10

TOSE'E KIMIA SA'ADAT has instruction sheets for several automatic analyzers. Instructions for many of them are available on request.

- **References:**

1-Karl J, Engel WD. Determination of Apolipoprotein A1 and B without sample dilution. Poster presented at the 57th meeting of the European Atherosclerosis Society, Lisbon and the IX European Congress of Clinical Chemistry, Cracow 1991.

2-Burtis CA, Ashwood ER. Tietz Fund. Of Clin. Chem. 5th ed. 30-54, 335-336, 462-494 and 972-973.

3-Consensus values of the Deutsche Gesellschaft fur Laboratoriums-medizin, the Deutsche Gesellschaft fur Klinische Chemie and the Verband der Diagnostica-Industrie.V. (VDGH). DG Klinische Chemie Mitteilungen 1995; 26:119-122.

4-Guder WG, Narayanan S, Wisser H, Zawta B. List of Analytes; Preanalytical Variables. Brochure in: Samples: From the patient to the Laboratory. Darmstadt: GIT Verlag, 1996.

Symbols:

The following symbols are used in the labelling of TOSE'E KIMIA SA'ADAT systems:

IVD	In Vitro Diagnostics	Σ	Contains sufficient for <n> tests
LOT	Batch Code		Temperature limit
REF	Catalogue No.		Consult instruction for use
	Expiry Date		Caution
	Date of Manufacture		Keep dry
	Manufactured by		This way up
	Biological Risks		Keep away from sunlight

Rev 01: Issued on 20 February 2023

ISO 13485:2016
ISO 9001:2015

IVD

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