COMPLEMENT C4

Diagnostic reagent for determination of Complement C4 concentration.
Liquid. Monoreagent. Store at +2/+8°C. For in Vitro Diagnostic Use (IVD). **Do not freeze.**

Ref No	Package	Ref No	Package	Ref No	Package	Ref No	Package
BB250	160 mL	LAB180	160 mL	M3B180	240 mL	TA180N	200 mL
BY181	350 mL	LAB181	160 mL	M4B180	300 mL	TA181N	100 mL
BZ2245	180 mL	LM448	160 mL	M4B181	150 mL	TA182N	25 mL
DMT180	333 mL	MAB180	300 mL	RD180	200 mL	TAB180	203 mL
HN422	200 mL	MAB181	150 mL	LM449	200 mL	TBAB180	150 mL
						8A181	350 mL

Changes made in the instructions for use are marked as grey.

INTENDED USE
The test is applied for the quantitative determination of C4 in serum and plasma.

GENERAL INFORMATION
There are about 30 serum complement proteins (15% of the globulin fraction), excluding cell surface receptors and regulatory proteins. Most of them are produced in the liver and reduced complement is a feature of severe liver failure.[1] The main function of the complement system is to collect effector phagocytes for opsonization and clearance of foreign pathogens and to trigger direct destruction of foreign organisms.[2] Complement proteins contribute to the acute phase response and high levels are seen in chronic untreated inflammation (e.g. rheumatoid arthritis). Once activated, complement is strongly proinflammatory. In fact, almost half of the proteins/receptors of the complement system play regulatory roles, reflecting the importance of controlling inappropriate activation.[1]

Overproduction and premature activation of the complement system may trigger an autoimmune response in which the body's immune system attacks its own tissues and organs. The resulting damage can be cumulative, as in rheumatoid arthritis (RA) and multiple sclerosis, or acute, as in the complete destruction of pancreatic islet cells in type 1 diabetes. Deficiencies in the production of complement factors can make a person susceptible to the emergence and spread of bacterial, fungal or viral infections. Infections such as bacterial and viral meningitis, streptococcal and staphylococcal sepsis and pneumonia are associated with a decrease in C4.[3-5] There is also a growing number of scientific studies on their pathophysiological contribution to the development of inflammatory, autoimmune, hematologic and nephrologic diseases; studies have also demonstrated etiologic links with various diseases, including cancer and neurodegenerative disorders.[6-9]

The complement system can be activated in 3 different ways: classical, alternative and lectin pathway.[10] C4 is a molecule involved in the classical pathway.

Routine measurement of serum C4 and C3 levels allows the exclusion or diagnosis of a number of pathogenic processes and can be useful in disease monitoring. Normal C4 levels effectively exclude the presence of mixed cryoglobulin. Treatment of the underlying process (immunosuppression, if autoimmune, chemotherapy, if there is clonal B-cell expansion) eliminates the cryoglobulin, allowing C4 levels to rise.[1]

Deficiencies of components of the classical pathway, including C4, lead to the development of autoimmune disorders [e.g. RA, systemic lupus erythematosus (SLE) and vasculitis] and predispose individuals to recurrent respiratory infections and infections caused by encapsulated organisms.[11] Reduction in C4 is common, but its complete absence is rare. Reduction or complete absence of C4 concentration can occur in immune complex diseases, SLE, autoimmune thyroiditis and juvenile dermatomyositis. It is elevated in systemic infections, chronic non-infectious inflammatory conditions (primarily chronic polyarthritis) and physiological conditions (pregnancy).[3,4,12,13] Excessive complement activation has also been described in many disease states such as hereditary/acquired angioedema, Alzheimer's syndrome, asthma, cerebral embolism, Crohn's disease, depression, glaucoma, Huntington's disease, myocardial infarction, various types of kidney disease [e.g. immune complex glomerulonephritis, hemolytic uremic syndrome (HUS), lupus nephritis, membranous nephritis, IgA nephropathy], malnutrition, resistant arterial hypertension, septicemia and serum sickness.[14]

TEST PRINCIPLE
Immunoturbidimetric method

In the presence of anti-human C4 antibodies, complement C4 in the sample precipitates. The turbidity of the antibody-antigen complex formed as a result of this precipitation is measured by absorbance reading at 340 nm wavelength and is proportional to the concentration of C4 in the sample.

REAGENT COMPONENTS

Reagent 1:

Goat anti-human C4 antibodies	
Imidazole buffer	≤ 0.11 mol/L
Sodium azide	≤ ≤ %0.1

REAGENT PREPARATION
Reagent is ready for use.

REAGENT STABILITY AND STORAGE

Reagents are stable at +2/+8˚C till the expiration date stated on the label which is only for closed vials.

Once opened vials are stable for 30 days at +2/+8°C in optimum conditions. On board stability is strongly related to auto analyzers' cooling specification and carry-over values.

Reagent stability and storage data have been verified by using Clinical and Laboratory Standards Institute (CLSI) EP25-A protocol.[15]

SAMPLE REQUIREMENTS

Serum and plasma are collected by standard procedure. Li heparin collection tubes should be preferred for plasma. Multiple sample freezing and thawing should be avoided.

Complement C4 activity stability in serum and plasma:

2 days at +20/+25 ºC
8 days at +2/+8 ºC
3 months at -20 °C

Annotation:

- Lipemic samples are not suitable for testing.

CALIBRATION AND QUALITY CONTROL

Calibration: The assay requires the use of a Protein Calibrator.

Protein Calibrator - Lyophilized
Ref.No: PC30
Ref.No: PC31
Ref.No: PC32 (For Olympus AU series)
Ref.No: PC33 (For Olympus AU series)

Calibration stability is 20 days. Calibration stability depends on the application characteristics and cooling capacity of the autoanalyzer used.

Control: Commercially available control material with established values determined by this method can be used. We recommend:

Protein Control Serum I-Lyophilized
Ref.No: PCN01
Ref.No: PCN02
Ref.No: PCN03 (For Olympus AU series)
Ref.No: PCN04 (For Olympus AU series)

Protein Control Serum II-Lyophilized
Ref.No: PCN05
Ref.No: PCN06
Ref.No: PCN07 (For Olympus AU series)
Ref.No: PCN08 (For Olympus AU series)

At least two level controls must be run once in every 24 hours. Each laboratory should determine its own quality control scheme and procedures. If quality control results are not within acceptable limits, calibration is required.

REFERENCE INTERVALS / MEDICAL DECISION LEVELS

Expected values[25]: 10 - 40 mg/dL

Each laboratory should investigate the transferability of the expected values to its own patient population and if necessary, determine its own reference range.

Reference interval has been verified by using CLSI EP28-A3c protocol.[16]

Unit Conversion:
mg/dL = 0.01 g/L

PERFORMANCE CHARACTERISTICS

Measuring Interval

According to CLSI EP34-ED1:2018, "Measuring Interval" refers to the interval where the analyte concentration is measured with intended accuracy in terms of medical and laboratory requirements without dilution, concentrating or any kind of pre-treatment that is between the analyte's lower limit of quantitation (LLoQ) and upper limit of quantitation (ULoQ).[17]

The determined analytic measuring interval for C4 is 1-90 mg/dL.

Detection Capability

Limit of Detection (LoD): 0.5 mg/dL

Limit of Quantitation (LoQ): 1 mg/dL

Note: LoQ values are based on Coefficient of Variation Percentage (CV) ≤ 20%.

LoD and LoQ values have been verified by using CLSI EP17-A2:2012 protocol.[18]

Linearity

This method shows measurement linearity in the activities up to 90 mg/dL. Autoanaylzer's auto-dilution system can be used if the concentrations have higher values. See device manual for further information.

For the manual dilution procedure, dilute the sample 1:5 using 0.90% isotonic. After this process, multiply the result of the reworked sample by the dilution factor. Do not report the sample result after dilution if it is marked as lower than the linear lower limit. Rerun with a suitable dilution.

Linearity Studies data have been verified by using CLSI EP06-A:2003 protocol.[19]

Precision

Running system has been developed according to 20x2x2 "The Single Site" protocol. Repeatability and Within-Laboratory Precision/Within-Device values have been obtained according to the running results.

According to the protocol in use, 2 separate runs per day have been made for 20 days (no obligation for being consecutive days). This protocol has been applied to each low and high samples separately and 80 results have been obtained for each one. Statistically, the results have been obtained using 2-factor Nested-ANOVA model.[20]

Repeatability (Within Run) and Repeatability (Day to Day) CV% values of C4 have been given in the table 1 and 2 respectively.

Table 1. C4 Repeatability (Within Run) results obtained from samples in two different concentrations

Mean Concentration	SD	CV%	n
28 mg/dL	0.40	1.42	80
83 mg/dL	0.86	1.03	80

Note: This working system has been named "Within-Run Precision" in the previous CLSI - EP05-A2 manual.[21]

Table 2. C4 Repeatability (Day to Day) results obtained from samples in two different concentrations

Mean Concentration	SD	CV%	n
28 mg/dL	0.69	2.47	80
83 mg/dL	2.53	3.05	80

Note: This working system has been named "Total Precision" in the previous CLSI - EP05-A2 manual.[21]

Prozone Effect: No prozone effect has been observed up to 150 mg/dL tested for C4.

Interference

Endogenous interferant and analyte concentrations that have been used in the C4 scanning tests has been determined according to "CLSI EP37-ED1:2018" and "CLSI EP07-ED3:2018" manuals.[22,23]

The total acceptable error rate, which is going to be used to detect whether the observed differential value obtained from C4 interference scanning test is appropriate, is determined as ±10%.[24]

In C4 test results, no significant interaction has been observed in the determined endogenous interferant and analyte concentrations or between interferants and analyte.

Hemoglobin	: ≤ 2.5 g/L
Bilirubin	: ≤ 20 mg/dL
Rheumatoid Factors	: ≤ 300 IU/mL
Lipemia	: ≤ 3.0 g/L

It should be noted that endogenous interferants, as well as various medicines and metabolites, anticoagulants (e.g. Heparin, EDTA, citrate, oxalate) and preservatives (e.g. sodium floride, iodoacetate, hydrochloride acide) such as additives, materials that may contact with samples during collection and processing (serum separator devices, sample collection containers and contents, catheters, catheter wash solutions, skin disinfectants, hand cleaners and lotions, glass washing detergents, powder gloves), dietary substances known to affect some specific tests (caffeine, beta-carotene, poppy seeds, etc.), or some substances present in a sample that cause foreign proteins (heterophilic antibodies, etc.), autoimmune response (autoantibodies, etc.), or due to malignancy (for example, interference by paraproteins with phosphate testing and indirect ion selective electrode methods) may show some negative effects that will cause various attempts and some misjudgements.[23]

These performance characteristics have been obtained using an autoanalyzer. Results may vary slightly when using different equipment or manual procedures.

WARNINGS AND PRECAUTIONS

IVD: For in Vitro Diagnostic use only.
Do not use expired reagents.
Reagents with two different lot numbers should not be interchanged.
For professional use.
Follow Good Laboratory Practice (GLP) guidelines.
Contains sodium azide.

CAUTION: Human source samples are processed with this product. All human source samples must be treated as potentially infectious materials and must be handled in accordance with OSHA (Occupational Safety and Health Administration) standards.

Danger

EUH032	:Releases a very toxic gas if contacts with acid.
H317	:May cause allergic skin reaction.

Precaution

P280	:Use protective gloves / clothes / glasses / mask.
P264	:Wash your hands properly after using.
P272	:Contaminated work clothes should not be allowed to be used outside of the workplace.

Intervention

P302+P352	:Wash with plenty of water and soap if it contacts with skin.
P333+P313	:Seek medical help if it irritates your skin or develops rash.
P362+P364	:Remove contaminated clothes and wash properly before using.

Disposal

P501	:Dispose the vials and contents according to the local regulations.

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SYMBOLS

IVD	In Vitro Diagnostic Medical Device
LOT	Lot Number
R1	Reagent 1
GTIN	Global Trade Item Number
REF	Reference Number
GLP	Good Laboratory Practices
FOR USE WITH	Identifies Products to Be Used Together
PRODUCT OF TURKEY	Product of Turkey
	Manufacturer
	Expiration Date
	Temperature Limits
	Consult Instructions for Use
	Caution
	Number of Tests