Complement C4 FS*

Diagnostic reagent for quantitative in vitro determination of complement component C4 in serum or plasma on photometric systems

Order Information

Cat. No.	Kit size					
1 1812 99 10 930	R1	4 x	20 mL	+ R2	2 x	8 mL
1 1812 99 10 935	R1	2 x	20 mL	+ R2	1 x	8 mL
5 9200 99 10 037		3 x	1 mL	TruCal Protein high		
5 9200 99 10 039		5 x	1 mL	TruCal Protein:		
	Calibrator set with 5 different levels					

Summary [1,2]

The complement system represents a group of at least 20 plasma proteins and several receptor proteins that interact in a regulated proteolytic cascade in order to destroy invading bacteria and prevent deposition of immuncomplexes. The activation results in decreased concentrations of C3 and/or C4 due to consumption of the intact proteins. The complement cascade can be activated by two different pathways. The classical pathway is activated by immuncomplexes or antibodies bound to bacteria or virus. The cascade starts with the binding of the C1q part of C1 to the Fc-part of the antibodies and it activates C3 by proteolysis of C4. The alternative pathway is activated independent of antibodies by microorganisms, Polysaccharides, autolysis of C3 or aggregated immunoglobulins. The alternative pathway does not need C4 protein.
Because C3 is common to both pathways lowered concentrations are indicating general complement activation. Lowered C3 values are found in inflammatory and infectious diseases especially in glomerulonephritis and SLE (Systemic Lupus erythematodes). Depending on the activated pathway C4 values can be lowered or stay normal. Isolated low values of C4 can occur in hereditary and acquired angioneurotic edema. Hereditary deficiency states of both complement factors have been reported.
C3 as well as C4 react as acute phase proteins. This increase due to an inflammatory process may mask moderately increased complement consumption.

Method

Immunoturbidimetric test

Principle

Determination of the C4 concentration by photometric measurement of antigen-antibody-reaction of antibodies to human C4 with C4 present in the sample.

Reagents

Components and Concentrations

R1:	TRIS	pH 7.5	100 mmol/L
	NaCl		320 mmol/L
R2:	TRIS	pH 8.0	100 mmol/L
	NaCl		300 mmol/L
	Anti-human C4 antibody (goat)		< 1%

Storage Instructions and Reagent Stability

The reagents are stable up to the end of the indicated month of expiry, if stored at 2 – 8°C and contamination is avoided. Do not freeze the reagents and protect reagents from light!

Warnings and Precautions

1. Reagent 1: Warning. H319 Causes serious eye irritation. P280 Wear protective gloves/protective clothing/eye protection/face protection. P305+P351+P338 If in eyes: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. P337+P313 If eye irritation persists: Get medical advice/attention.
2. The reagents contain sodium azide (0.95 g/L) as preservative. Do not swallow! Avoid contact with skin and mucous membranes!
3. Reagent 2 contains animal material. Handle the product as potentially infectious according to universal precautions and good clinical laboratory practices.
4. In very rare cases, samples of patients with gammopathy might give falsified results [6].
5. Please refer to the safety data sheets and take the necessary precautions for the use of laboratory reagents. For diagnostic purposes, the results should always be assessed with the patient's medical history, clinical examinations and other findings.
6. For professional use only!

Waste Management

Please refer to local legal requirements.

Reagent Preparation

The reagents are ready to use.

Materials required but not provided

NaCl solution 9 g/L
General laboratory equipment

Specimen [3]

Serum, heparin plasma or EDTA plasma

During storage of serum the C3 and C4 proteins slowly degrade into C3c resp. C4 fragments (fragmentation is inhibited by EDTA). These fragments still contain the reactive epitopes and may even display higher signals than the intact protein. Depending on the conditions of this aging process, fresh serum samples may show up to 30% lower C3 values than samples stored at 2 – 8°C for 8 days. The fragmentation of C4 is much slower than for C3 and only 15% lower values can be observed under similar storage conditions.

Discard contaminated specimens.

Assay Procedure for Analyzers

Application sheets for automated systems are available on request.

Wavelength	340 nm
Optical path	1 cm
Temperature	37°C
Measurement	Against reagent blank

	Blank	Sample or calibrator
Sample or calibrator	-	10 µL
Dist. water	10 µL	-
Reagent 1	350 µL	350 µL
Mix, incubate for 3 – 5 min., read absorbance (A1), then add:		
Reagent 2	70 µL	70 µL
Mix, incubate for 5 min., read absorbance (A2).		

$\Delta A = (A2 - A1)$ sample or calibrator

Calculation

The concentration of C4 in unknown samples is derived from a calibration curve using an appropriate mathematical model such as logit/Log or spline. The calibration curve is obtained with 5 calibrators at different levels and NaCl solution (9 g/L) for determination of the zero value.
Stability of calibration: 4 weeks

Calibrators and Controls

For the calibration of automated photometric systems, DiaSys TruCal Protein calibrator set or the TruCal Protein high calibrator is recommended. The assigned values of the calibrators have been made traceable to the ERM®-DA470k/IFCC Reference Material. DiaSys TruLab Protein control should be assayed for internal quality control. Each laboratory should establish corrective action in case of deviations in control recovery.

	Cat. No.	Kit size
TruLab Protein Level 1	5 9500 99 10 046	3 x 1 mL
TruLab Protein Level 2	5 9510 99 10 046	3 x 1 mL

Performance Characteristics

Measuring Range

The test has been developed to determine concentrations of C4 within a measuring range from 0.6 – 90 mg/dL (0.006 – 0.9 g/L) – depending on the highest calibrator. When values exceed this range samples should be diluted 1 + 1 with NaCl solution (9 g/L) and the result multiplied by 2.

Prozone Limit

No prozone effect was observed up to an C4 value of 180 mg/dL (1.8 g/L).

Specificity/Interferences

Due to its antibodies, DiaSys Complement C4 is a specific immunoassay for human C4. No interference was observed by conjugated and unconjugated bilirubin up to 60 mg/dL, hemoglobin up to 1000 mg/dL, lipemia up to 2000 mg/dL triglycerides and RF up to 1200 IU/mL.
Interference by monoclonal gammopathies was not observed up to 6400 mg/dL IgA, 4100 mg/dL IgM and 6400 mg/dL IgG. For further information on interfering substances refer to Young DS [5].

Sensitivity/Limit of Detection

The lower limit of detection is 0.6 mg/dL (0.006 g/L).

Imprecision

Intra-assay precision n = 20	Mean [mg/dL]	SD [mg/dL]	CV [%]
Sample 1	27.2	0.895	3.29
Sample 2	42.4	1.64	3.87
Sample 3	55.2	1.34	2.43

Inter-assay precision n = 20	Mean [mg/dL]	SD [mg/dL]	CV [%]
Sample 1	28.6	0.959	3.35
Sample 2	39.7	0.965	2.43
Sample 3	52.3	1.62	3.10

Total precision according to protocol EP-5 of the NCCLS (National Committee of Clinical Laboratory Standards):

Total precision n = 80	Mean [mg/dL]	SD [mg/dL]	CV [%]
Sample 1	28.9	0.983	3.40
Sample 2	40.2	1.073	2.67

Method Comparison

A comparison of DiaSys Complement C4 FS (y) to an immunoturbidimetric test (x) using 107 samples gave following results:
y = 0.97 x - 0.053 mg/dL; r = 0.984

A comparison of DiaSys Complement C4 FS (y) to a nephelometric test (x) using 92 samples gave following results:
y = 1.12 x - 0.21 mg/dL; r = 0.975

Reference Range [4]

10 – 40 mg/dL (0.1 – 0.4 g/L)

Each laboratory should check if the reference ranges are transferable to its own patient population and determine own reference ranges if necessary.

Literature

1. Thomas L. Clinical Laboratory Diagnostics. 1st ed. Frankfurt: TH-Books Verlagsgesellschaft; 1998. p. 794–806.
2. Johnson AM, Rohlfs EM, Silverman LM. Proteins. In: Burtis CA, Ashwood ER. editors. Tietz textbook of clinical chemistry. 3rd ed. Philadelphia: W. B. Saunders Company; 1999. p. 502-7.
3. Okumura N, Nomura M, Tada T et al. Effects of sample storage on serum C3c assay by nephelometry. Clin Lab Sci 1990; 3(1): 54–57.
4. Dati F, Schumann G, Thomas L, Aguzzi F, Baudner S, Bienvenu J et al. Consensus of a group of professional societies and diagnostic companies on guidelines for interim reference ranges for 14 proteins in serum based on the standardization against the IFCC/BCR/CAP reference material (CRM 470). Eur J Clin Chem Clin Biochem 1996;34:517-20.
5. Young DS. Effects of Drugs on Clinical Laboratory Tests. 5th ed. Volume 1 and 2. Washington, DC: The American Association for Clinical Chemistry Press 2000.
6. Bakker AJ, Mücke M. Gammopathy interference in clinical chemistry assays: mechanisms, detection and prevention. ClinChemLabMed 2007;45(9):1240–1243.

Manufacturer

IVD CE

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