Complement C4 (C4c)

Immunoturbidimetry

Cat.No	Package Size
813 000	5 x 20 ml R1 / 1 x 20 ml R2
813 031 Hit I BC	4 x 20 ml R1 / 2 x 8 ml R2

General Information

C3 and C4 are part of the complement system , a group of plasma proteins and receptor proteins that interact in a proteolytic cascade to destroy bacteria and prevent deposition of immuncomplexes. The result is decrease of C3 and C4. The complement cascade can be activated by two different pathways. Depending on the activation of either the one or the other, C4 is reduced or it stays normal.
Decreasing only C4 indicates e.g.angioneurotical edema.
Note: C3 and C4 react also as acute phase proteins. An increase due to an inflammatory process may mask a moderately increased complement consumption.

Method and Principle

Immunoturbidimetric test with endpoint determination of the concentration of C4 through photometric measurement of antigen-antibody-reaction between antibodies to human C4 and C4 present in the sample.

Reagents

Components (concentrations in the test)

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R1:	Phosphate Buffer	pH 7.5	100 mmol/l
	NaCl		320 mmol/l
	Polyethylenglycol (PEG)		
	Detergents, stabilizers		
R2:	Phosphate Buffer	pH 8.0	100 mmol/l
	NaCl		320 mmol/l
	Anti-human C4 antibody (goat) with stabilizers		

Storage / Stability

Reagents R1 and R2 are stable up to the expiry date, if stored at 2 – 8 °C and contamination is avoided. Do not freeze the reagents!

Warnings and Precautions

Reagents contain sodium azide (0.95 g/l). Do not swallow! Avoid contact with skin and mucous membranes!

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.

Samples

Serum, heparin or EDTA plasma.
Note:
During storage of serum C3 and C4 proteins slowly fragment into C3c resp. C4c components (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 15 % lower values than samples stored at 2 – 8 °C for 8 days .(The fragmentation of C4 is much slower than for C3 and so only 15 % lower values can be observed under similar storage conditions, as compared to C3).
Discard contaminated samples!

Reference Range (according to IFCC)

9 - 39 mg/dl
In case of fresh samples lower reference ranges are expected.
Each laboratory should establish own reference ranges in order to reflect its specific working conditions.

Assay Procedure

Wavelength	340 nm
Cuvette	1 cm
Temperature	37 °C
Measurement	against reagent blank

	Blank	Sample / calibrator
Sample / calibrator	-	5 µl
Dist. water	5 µl	-
R 1	250 µl	250 µl
Mix, incubate for 3 – 5 min, read absorbance (A1), then add:		
R 2	50 µl	50 µl
Mix, incubate for 5 min, read absorbance (A2).		

ΔA = [(A2 – A1) *sample or calibrator*] - [(A2 – A1) *blank*]

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

Applications for automated systems are available on request.

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