Cromatest

LINEAR

C4 at CE

Complement C4

Turbidimetric method

REF 3171005
1 x 50 mL
CONTENTS
R1.Reagent 1 x 50 mL

For *in vitro* diagnostic use only

PRINCIPLE

C4 at is a quantitative turbidimetric assay for the measurement of the component complement C4 in human serum or plasma.
Anti-human C4 antibodies form insoluble complexes when mixed with samples containing C4. The scattering light of the immunocomplexes depends of the C4 concentration in the patient sample, and can be quantified by comparison from a calibrator of known C4 concentration.

REAGENTS COMPOSITION

R1 **C4 at**. Goat antibodies anti-human C4, tris buffer 20 mmol/L, pH 8.2. Sodium azide 0.95 g/L.

Plasma Protein Multicalibrator. Protein Calibrator. Optional . Ref: 3910005.

Precautions: The reagent contains sodium azide 0.95 g/L. Avoid any contact with skin or mucous.

STORAGE AND STABILITY

1. Store at 2-8ºC.
   The reagent is stable until the expiry date stated on the label, when stored tightly closed at 2-8ºC and contaminations are prevented during their use. Does not use the reagent after the expiry date.
2. Presence of particles, turbidity and/or the absorbance of blank reagent > 0.3 at 340 nm are sign of deterioration.

REAGENT PREPARATION

R1 Ready to use.

Calibration curve. Dilute the Plasma Protein Calibrator in NaCl 9 g/L as follow:

Dilution	1	2	3	4	5	6
Calibrator (µL)	--	10	25	50	75	100
NaCl 9 g/L (µL)	100	90	75	50	25	--
Factor	0	0.1	0.25	0.5	0.75	1.0

Multiply the concentration of the C4 Protein Calibrator by the corresponding factor to obtain the C4 concentration of each dilution.

SAMPLES

Fresh serum and EDTA or heparinized plasma.
C4 in serum or plasma is stable 7 days at 2-8ºC or 3 months at –20ºC.
Samples with presence of fibrin should be centrifuged before testing. Hemolyzed or lipemic samples are not suitable for testing.

INTERFERENCES

Bilirubin (10 mg/dL) and rheumatoid factors (400 UI/mL) do not interfere. Hemoglobin (4 g/L) and lipemia (6 g/L) may affect the results. Other substances may interfere[5].

MATERIAL REQUIRED

- Thermostatic bath at 37ºC.
- Spectrophotometer or photometer thermostatable at 37ºC capable to read at 340 ± 20 nm.
- Cuvettes with 1cm pathlength.
- Pipettes to measure reagent and samples.

PROCEDURE

1. Prewarm the reagent and the photometer (cuvette holder) to 37ºC.
2. Using distilled water zero the instrument at 340 nm.
3. Pipette into a cuvette:

Sample / Calibrator	25 µL
Reagent (R1)	1.0 mL

4. Mix well and insert the cuvette into the photometer. Record the absorbance (A) after 2 minutes of the sample or calibrator addition.

CALCULATION

Plot the different absorbance values (A) against the C4 concentration of each calibrator dilution. C4 concentration in the sample is calculated by interpolation of its (A) value in the calibration curve.

LINEAR CHEMICALS, S.L.U. Joaquim Costa 18 2ª planta. 08390 Montgat (Barcelona) SPAIN
Telf. (+34) 934 694 990; E-mail: info@linear.es ; website: www.linear.es NIF-VAT:B60485687

REFERENCE VALUES

Adults[3]: 10 – 40 mg/dL
Newborn[4]: 13 – 38 mg/dL
It is recommended that each laboratory establishes its own reference range.

QUALITY CONTROLS

To ensure adequate quality control (QC), each run should include a set of controls (normal and abnormal) with assayed values handled as unknowns.

REF **3915010 PLASMA PROTEIN CONTROL N-I**
Normal level. Assayed.

REF **3915015 PLASMA PROTEIN CONTROL N-II**
Abnormal level. Assayed.

Each laboratory should establish its own Quality Control scheme and corrective actions if controls do not meet the acceptable tolerances.

CLINICAL SIGNIFICANCE

C4 is the complement component essential for classical pathway activation. Most individuals with C4 deficiency do not have problems with infection, suggesting that the alternative pathway can compensate for the lack in the classical pathway activation in removal of bacterial agents. Hepatic cells synthesize C4, although in less proportion may be synthesized by monocytes and other tissues.

Increased and decreased levels of C4 both have clinical significance.

Increased levels are closely related with acute-phase response (trauma, inflammatory process).

Decreased levels are related with genetic deficiency (autoimmune or collagen vascular disease, particularly Systemic Lupus Erythematosus), or acquired deficiency as a consequence of the consumption in immunocomplexes formation, autoimmune hemolytic anemia and autoimmune nephritis.

ANALYTICAL PERFORMANCE

- ***Linearity limit.*** Up to 100 mg/dL, under the described assay conditions. Samples with higher concentrations should be diluted 1/5 en ClNa 9 g/L and retested again.
- ***Detection limit.*** Values less than 0.5 mg/dL give non-reproducible results.
- ***Analytical sensitivity.*** Using this reagent and method an ΔA of 9.34 mA at 340 nm is equivalent to 1 mg/dL of C4 at a concentration of 47.6 mg/dL.
- ***Prozone effect.*** Prozone effect is not observed up to 200 mg/dL.
- ***Precision.***

mg/dL	Within-run		Between-run	
Mean	19.9	37.6	19.9	37.6
SD	0.6	0.9	0.7	1.8
CV%	3.1	2.3	3.6	4.7
N	10	10	10	10

Instrument: Cobas Mira

- ***Accuracy:*** Results obtained with this reagent did not show systematic differences when compared with commercial reagents of similar characteristics. Details of comparison are available on request.

NOTES

1. This method may be used with different instruments. Any application to an instrument should be validated to demonstrate that results meet the performance characteristics of the method. It is recommended to validate periodically the instrument. Contact to the distributor for any question on the application method.

2. The linearity limit depends on the sample/reagent ratio, as well as the analyzer used. It will be higher by decreasing the sample volume, although the sensitivity of the test will be proportionally decreased.

3. Clinical diagnosis should not be made on findings of a single test result, but should integrate both clinical and laboratory data.

REFERENCES

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4. Tietz *Textbook of Clinical Chemistry, 3rd* Ed. Burtis CA, Ashwood ER. WB Saunders Co., (1999).
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