AU

Instructions For Use

C4
C4

REF OSR6160 4 x 10 mL R1, 4 x 8 mL R2

For *in vitro* diagnostic use only.

PRINCIPLE

INTENDED USE

Immuno-turbidimetric test for the quantitative determination of C4 (Complement 4) in human serum and plasma on Beckman Coulter analysers.

SUMMARY AND EXPLANATION

Reference[1,2]

The Complement system consists of about 20 plasma proteins as well as receptors on blood cells that play an important role in inflammation by facilitating phagocytosis through opsonisation, lysing foreign cells, increasing vascular permeability and attracting phagocytes. Activation or consumption of complement occurs in a number of disorders, particularly those involving immune complex deposition e.g. SLE, mixed cryoglobulinaemia and some forms of vasculitis, however this may be compensated for in part by the synthesis of acute phase reactants. C3 comprises about 30% of the total plasma concentration of complement components and is consumed by activation of both the classical and alternative pathways. C4 levels fall only as a consequence of classical pathway activation, therefore if hypocomplementemia is present, measurement of both C3 and C4 can determine whether the classical or alternative pathway has been activated.

METHODOLOGY

When a sample is mixed with R1 buffer and R2 antiserum solution, human C4 reacts specifically with anti-human C4 antibodies to yield insoluble aggregates. The absorbance of these aggregates is proportional to the C4 concentration in the sample.

SPECIMEN

TYPE OF SPECIMEN

Serum and EDTA or heparinised plasma.

SPECIMEN STORAGE AND STABILITY

Stable in serum and plasma for 8 days when stored at 2...8°C and 2 days when stored at 15...25°C.[3]

Strongly lipemic samples should be avoided.

REAGENTS

WARNING AND PRECAUTIONS

Exercise the normal precautions required for handling all laboratory reagents.

Dispose of all waste material in accordance with local guidelines.

This product contains material of animal origin. The product should be considered as potentially capable of transmitting infectious diseases.

REACTIVE INGREDIENTS

Final concentration of reactive ingredients:

Tris buffer (pH 7.2)	62 mmol/L
Polyethylene glycol 6000	1.6% w/v
Goat anti-C4 antibodies	Variable
Preservative	

The concentrations of the reactive components of the reagents shown on the kit label are the actual concentrations in the individual R1/R2 vials. The reagent composition which is shown in the Instructions For Use is the final concentration of these components in the reaction cuvette after addition of R1, Sample, and R2.

⚠ CAUTION

Sodium azide preservative may form explosive compounds in metal drain lines. See NIOSH Bulletin: Explosive Azide Hazard (8/16/76).
To avoid the possible build-up of azide compounds, flush wastepipes with water after the disposal of undiluted reagent. Sodium azide disposal must be in accordance with appropriate local regulations.

GHS HAZARD CLASSIFICATION

C4 R1	WARNING	
	H316	Causes mild skin irritation.
	P332+P313	If skin irritation occurs: Get medical advice/attention.
		Tris(hydroxymethyl)– aminomethane 1 - 5%

SDS	Safety Data Sheet is available at beckmancoulter.com/techdocs

REAGENT PREPARATION

The reagents are ready for use and can be placed directly on board the instrument.

REAGENT STORAGE AND STABILITY

The reagents are stable, unopened, up to the stated expiry date when stored at 2…8°C. Once open, reagents stored on board the instrument are stable for 90 days.

CALIBRATION

CALIBRATOR REQUIRED

Serum Protein Multi-Calibrator Cat. No. ODR3021.

The calibrator values are traceable to the IFCC (International Federation of Clinical Chemistry) standard CRM 470.

Recalibrate the assay every 90 days or when the following occur:

Change in reagent lot or significant shift in control values;

Major preventative maintenance was performed on the analyser or a critical part was replaced.

Following calibration, the resulting curve should be visually reviewed for acceptability, on the Beckman Coulter analyser, using the software options to access the Calibration Monitor. Quality control procedures should be undertaken immediately following calibration in accordance with good laboratory practice.

QUALITY CONTROL

ITA Control Sera ODC0014, ODC0015 and ODC0016 or other control materials with values determined by this Beckman Coulter system may be used.

Each laboratory should establish its own control frequency however good laboratory practice suggests that controls be tested each day patient samples are tested and each time calibration/blanking is performed.

The results obtained by any individual laboratory may vary from the given mean value. It is therefore recommended that each laboratory generates analyte specific control target values and intervals based on multiple runs according to their requirements. These target values should fall within the corresponding acceptable ranges given in the relevant product literature.

If any trends or sudden shifts in values are detected, review all operating parameters.

Each laboratory should establish guidelines for corrective action to be taken if controls do not recover within the specified limits.

TESTING PROCEDURE(S)

Refer to the appropriate Beckman Coulter AU analyser User Guide/Instructions For Use (IFU) for analyser-specific assay instructions for the sample type as listed in the Intended Use statement. The paediatric application is suitable for use with small volume serum/plasma samples.

CALCULATIONS

The Beckman Coulter analyzers automatically compute the C4 concentration of each sample.

REPORTING RESULTS

REFERENCE INTERVALS

Reference[4,5]

Adults and children	0.1 – 0.4 g/L (10 – 40 mg/dL)

Expected values may vary with age, sex, sample type, diet and geographical location. Each laboratory should verify the transferability of the expected values to its own population, and if necessary determine its own reference interval according to good laboratory practice. For diagnostic purposes, results should always be assessed in conjunction with the patient's medical history, clinical examinations and other findings.

PROCEDURAL NOTES

LIMITATIONS

Samples with extremely abnormal optical characteristics, especially turbidity, may produce atypical results.

INTERFERENCES

Results of studies conducted to evaluate the susceptibility of the method to interference were as follows:

Icterus:	Interference less than 10% up to 40 mg/dL or 684 µmol/L bilirubin
Haemolysis:	Interference less than 5% up to 5 g/L haemoglobin
Lipemia:	Interference less than 10% up to 500 mg/dL Intralipid

Refer to Young[6] for further information on interfering substances.

PERFORMANCE CHARACTERISTICS

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Data contained within this section is representative of performance on Beckman Coulter systems. Data obtained in your laboratory may differ from these values.

LINEARITY

The test is linear within a concentration range of 0.08 – 1.50 g/L (8 – 150 mg/dL).

SENSITIVITY

The lowest detectable level in serum on an AU480 analyser was estimated at 0.001 g/L.

The lowest detectable level represents the lowest measurable level of C4 that can be distinguished from zero. It is calculated as the absolute mean plus three standard deviations of 20 replicates of an analyte free sample.

METHODS COMPARISON

Patient serum samples were used to compare this C4 OSR6160 assay on the AU600 against another commercially available C4 assay. Results of linear regression analysis were as follows:

y = 1.063x - 0.05	r = 0.972	n = 81	Sample range = 0.08 – 1.01 g/L

PRECISION

The following data was obtained on a DxC 700 AU using 3 serum pools analysed over 20 days.

n=80	Within-run		Total	
Mean g/L	**SD**	**CV%**	**SD**	**CV%**
0.14	0.002	1.16	0.004	2.52
0.29	0.003	0.99	0.004	1.52
1.41	0.009	0.66	0.013	0.95

ADDITIONAL INFORMATION

DxC 700 AU requires that each reagent application has a standard format of abbreviated Closed Test Name. This Closed Test Name is required to allow automated loading of the calibrator information for each application as part of the DxC 700 AU Closed System. Refer to the table below for the Closed Test Name assigned to each application for this assay.

Test Name	Description
C-41G	C4 (Serum)
C-41GP	C4 (Serum Paediatric)

Setting Sheet Footnotes

User defined

† Beckman Coulter Serum Protein Multi-Calibrator Cat. No: ODR3021

* Values set for working in SI units (g/L). To work in mg/dL multiply by 100.

REVISION HISTORY

Added new languages

Preceding version revision history

IFU updated to add Vietnamese language.

Revised GHS section

Updated Additional Information section

REFERENCES

1. Ismail AA, Snowden N. Autoantibodies and specific serum proteins in the diagnosis of rheumatological disorders. Ann Clin Biochem 1999;36:565-578.

2. Thomas L. The complement system. In:Thomas L, ed. Clinical laboratory diagnostics. Use and assessment of clinical laboratory results. Frankfurt/Main: TH-Books Verlagsgesellschaft, 1998:794-806.

3. Ehret W, Heil W, Schmitt Y, Töpfer G, Wisser H, Zawta B, et al. Use of anticoagulants in diagnostic laboratory investigations and stability of blood, plasma and serum samples. WHO/DIL/LAB/99.1 Rev.2:27pp.

4. Baudner S, Dati F. Standardization of the measurement of 14 proteins in human serum based on the new IFCC/BCR/CAP international reference material CRM 470. J Lab Med 1996;20:145-152.

5. Painter PC, Cope JY, Smith JL. Reference information for the clinical laboratory. In: Burtis CA, Ashwood ER, eds. Tietz textbook of clinical chemistry. Philadelphia:WB Saunders Company, 1999;1807pp.

6. Young DS. Effects of drugs on clinical laboratory tests, 5th ed. AACC Press, 2000.

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