



EIAgen C3d Circulating Immune Complex Kit

[REF] 11C3D-100C



96 tests

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FOR IN VITRO DIAGNOSTIC USE ONLY



Store at 2...8 °C



SYMBOLS USED ON LABELS

[IVD]	In vitro diagnostic medical device (In vitro diagnostic use)
[LOT]	Lot number
[REF]	Catalogue Code
[MT_PLATE]	Microplate
[CONJ HRP]	Conjugate
[CAL_1]	Calibrator 1
[CAL_2]	Calibrator 2
[RCNS]X_ml_	Reconstitute with X ml
[CONTROL +]	Positive Control
[CONTROL -]	Negative Control
[SOLN TMB]	Substrate (TMB)
[DILSPE]	Sample Diluent
[BUF WASH 20x]	Washing Buffer 20X
[SOLN STOP]	Stop Solution



Expiry date (Use by...)



Temperature limitation (store at 2...8°C)



Number of tests



Keep away from sunlight



Manufactured by



Attention, See Instructions For Use



Biological risk

1.0 INTENDED USE

The EIAgen C3d-CIC kit is an enzyme-linked immunosorbent assay method for the semi-quantitative determination of specific C3d-containing circulating immune complexes in human serum. The results of the C3d-CIC assay can be used as an aid in the diagnosis and monitoring of immune dysfunction. Levels of these complexes are one indicator in a multi-factorial diagnostic regime.

2.0 SUMMARY AND EXPLANATION OF THE TEST

Circulating immune complexes are present in many individuals with Systemic Lupus Erythematosus (SLE) and Rheumatoid Arthritis (RA), especially with any of the vasculitides complications. Levels of CICs have been reported to show correlation with disease activity in that higher levels are reported during active phases of the disease.

Many tests have been developed for the detection of CICs, including PEG precipitation and radial immunodiffusion. No single procedure appears to detect all types of CIC however, those procedures, such as the Raji cell assay and C3d solid phase assay, which detect CICs containing fragments of C3 (i.e. C3d) appear to detect clinically relevant events.

The EIAgen test system for C3d circulating immune complexes detects immune complexes containing both C3d and IgG. The concentration is expressed as µg/ml heat aggregated human globulin (HAG) equivalents.

The EIAgen test range also includes a kit for C1q circulating immune complexes which detects immune complexes containing both C1q and IgG.

3.0 PRINCIPLE OF THE TEST

The EIAgen assay is a solid phase immunosorbent assay (ELISA) in which the analyte is indicated by a colour reaction of an enzyme and substrate. The EIAgen wells are coated with anti-C3d monoclonal antibody .

On adding diluted serum to the wells the C3d-containing CICs present bind to the antibody. After incubating at room temperature and washing away unbound material, horseradish peroxidase conjugated anti-IgG monoclonal antibody is added, which binds to the immobilised complexes.

Following further incubation and washing, tetra-methyl benzidine substrate (TMB) is added to each well. The presence of the complex turns the substrate to a dark blue colour. Addition of the stop solution turns the colour to yellow.

The colour intensity is proportional to the amount of immune complexes present in the original serum sample.

4.0 CONTENT OF THE KIT

4.1 MICROPLATE [MT_PLATE]
(code 43PIA) One microplate is supplied which contains 12 strips of 8 breakpart wells. The wells are coated with mouse anti-C3d antibody

4.2 CONJUGATE [CONJ|HRP]
(code 43HRP) One vial containing 15ml of ready-to-use HRP conjugate. Conjugate contains 0.05% Proclin 300. Conjugates are color coded pink.

4.3 CALIBRATORS
(code 43CAL1-2) Six vials containing lyophilised calibrators (3 x level 1; 3 x level 2). For calibrator concentration see label on vials. These calibrators contain human antisera and must be reconstituted, diluted and stored in accordance with the instruction in the 'Reagent Preparation' section.

Calibrator	symbol	code
Calibrator 1 (level 1)	[CAL_1]	43CAL1
Calibrator 2 (level 2)	[CAL_2]	43CAL2

4.4 POSITIVE CONTROL [CONTROL|+]
(code 43POS) One vial containing 0.45 ml of concentrated positive control which contains human antisera and 0.09% sodium azide as a preservative.

4.5 NEGATIVE CONTROL [CONTROL|-]
(code 43NEG) One vial containing 0.45 ml of concentrated negative control which contains normal human serum and 0.09% sodium azide as a preservative.

The controls (positive and negative) are provided in a concentrated form and should be diluted 1/5 with sample diluent buffer before use. Prepare fresh control dilutions before each assay run. Vortex all samples and controls before testing.

4.6 SUBSTRATE (TMB) [SOLN|TMB]
(code TMBC) One vial containing 15 ml of ready-to-use tetra-methylbenzidine (TMB) substrate.

4.7 SAMPLE DILUENT [DILSPE]

(code 43SD) One bottle containing 100 ml of ready-to-use sample diluent buffer. The buffer includes 0.09% Sodium azide. Sample diluent buffer is color coded blue.

4.8 WASHING BUFFER 20X [BUF|WASH|20x]

(code TLAVC) One bottle containing 50 ml of wash buffer concentrate. Wash buffer concentrate contains 0.06% Proclin 300.

Dilute the whole content of the bottle to one liter with distilled or deionized water. Mix well before use. Store this solution at 2...8 °C if it is not to be used immediately. The diluted wash buffer is stable at 2...8 °C for one week.

4.9 STOP SOLUTION [SOLN|STOP]

(code STOPC) One bottle containing 20 ml of 0.25M H₂SO₄ stop solution.

The MSDS is available upon request of laboratory personnel.

5.0 STORAGE AND STABILITY AFTER THE FIRST OPENING

- Store kit components at 2-8 °C and do not use after the expiry date on the box outer label. Before use all components should be allowed to warm up to ambient temperature (18-25 °C).
- After use, the plate should be resealed, the bottle caps replaced and tightened and the kit stored at 2-8 °C.
- Refer to "Reagent Preparation" for instructions for the calibrators.
- The opened kit should be used within three months.
- Reconstituted calibrators are stable at 2-8°C for up to 5 days. Dilute the reconstituted calibrators 1/5 with sample diluent buffer prior to testing. These calibrators must now be stored at 2-8°C and used within 48 hours. **Reconstituted calibrators must be not be frozen.**

6.0 MATERIALS AND EQUIPMENT REQUIRED BUT NOT PROVIDED

- Distilled or deionized water.
- Wash bottle, automated or semi-automated microwell plate washing system.
- Rack for sample dilution.
- Micropipettes including multichannels capable of accurately delivering 5-1000µl (less than 3% cv).
- Reagent reservoirs for multichannel pipettes.
- One-liter graduated cylinder.
- Disposal basins and 0.5% sodium hypochlorite (50 ml bleach in 950 ml water).
- Single or dual wavelength microplate reader with 450 nm filter. If dual wavelength is used, set the reference filter to 600 - 650 nm.
- Paper towels, pipette tips and timer

7.0 WARNINGS OR PRECAUTIONS

7.1 SAFETY PRECAUTIONS

- All reagents in this kit are for in vitro diagnostic use only
- Only experienced laboratory personnel should use this test and handling should be agreement with GLP.
- Operators should wear gloves and protective clothing when handling any patient sera or serum based products.
- Reagents contain preservatives which may be toxic if ingested. Do not pipette by mouth. Avoid contact of reagents or patient samples with skin or mucous membranes. If contact occurs, immediately flush with large quantities of water.
- Avoid splashing or creation of aerosols.
- Reusable glassware must be thoroughly washed and rinsed so that it is free of all detergents.
- Sera used in the preparation of the calibrators and controls have been tested for the presence of antibodies to Human Immunodeficiency Virus (HIV 1 and 2), as well as for Hepatitis B Surface Antigen (HBsAg) and HCV and found to be negative. All material is tested with FDA approved assays. Because no test method can offer complete assurance that HIV, HBsAg or other infectious agents are absent, it is recommended that human serum based products be handled with the same precautions used for patient specimens.
- Dispose of reagent solutions containing sodium azide and thimerosal as preservatives according to all local, state and national regulations. To dispose of reagents containing azide, flush away using copious amounts of water. Dispose with caution as sodium azide can form explosive compounds on prolonged contact with lead or copper piping.

7.2 TECHNICAL PRECAUTIONS

A. Correct use of reagents and proper pipetting

- The performance data represented here were obtained using specific reagents listed in the package insert. Do not use reagents from other manufacturers in the kits.
- Do not use reagents from other EIAgen kits with this kit. Do not mix reagents from different kit lots.
- Do not dilute or adulterate the kit reagents, unless directed by the kit protocol.
- Do not use the substrate solution if it has begun to turn blue.
- Do not use heat-inactivated serum.
- Microplate washing is important. Improperly washed wells will give erroneous results

B. Adherence to assay procedure and specifications

The obtained values have to be always compared to the ones reported in QC sheet. Do not use the kit to determine values outside the range indicated in the IFU.

The test protocol must be followed strictly. Observe the indicated incubation times and temperature and the washing procedure. These are critical steps.

Include the positive and negative controls in every test run to monitor for reagent stability and correct assay performance. Please refer also to section 12.0_Quality Control.

8.0 SPECIMEN COLLECTION AND STORAGE

Use serum in this procedure.

It is most important to preserve the chemical integrity of a blood specimen from the moment it is collected until it is assayed. Obtain patient samples by non-traumatic venipuncture, using a vacuum tube or sterile syringe. If a syringe is used, transfer the blood immediately to a vacuum tube (plain red-top or serum separator).

Allow samples to clot at room temperature (18-25 °C) for at least 20-30 minutes, until the clot just begins to retract. Spin the sample in a centrifuge. Immediately following the centrifugation, transfer the cell-free serum to a tightly stoppered storage bottle.

Do not use sera samples showing signs of haemolysis. If it is necessary to store a sample prior to analysis, it is recommended that, for a period of up to 72 hours, store the sample in a sealed container at 2-8 °C. Freeze samples at -20 °C if longer storage is required. Avoid repeat freeze-thawing.

9.0 ASSAY PROCEDURE

9.1 REAGENT PREPARATION

Bring all reagents to room temperature (18-25 °C).

- Select sufficient microwells for the test. Remove protective covering and select sufficient wells to accommodate the patient samples, calibrators and assay controls. Each sample is recommended to be tested in duplicate.

Wash plate three times with diluted wash buffer immediately prior to commencing the assay (remove excess wash buffer by tapping on absorbent paper but do not allow the plate to dry out).

- Dilute all serum samples and assay controls 1/5 in sample diluent by adding 50µl to 200µl sample diluent.

- CALIBRATOR RECONSTITUTION: reconstitute one vial each (level 1 and 2) lyophilized calibrator by adding 0.15 ml of deionized water to each vial. Gently re-suspend the powder. Allow the reconstituted calibrators to sit at room temperature (18-25°C) for at least 10 minutes before use. Do not mix or vortex vigorously as this may result in denaturation of serum IgG and result in abnormally high results. Reconstituted calibrators are stable at 2-8°C for up to 5 days. Dilute the reconstituted calibrators 1/5 with sample diluent buffer prior to testing. These calibrators must now be stored at 2-8°C and used within 48 hours. **Reconstituted calibrators must be not be frozen.**

9.2 PIPETTING AND INCUBATION STEPS

A. Pipette 100µl of the calibrators, diluted control or diluted patient sample into the wells. Dispense 100µl of sample diluent as zero calibrator in the first well.

B. Incubate the wells at room temperature (18-25 °C) for 30 minutes.

C. Wash the wells three times as described in section 9.3_ PROCEDURAL NOTES

D. Add 100µl of ready-to-use conjugate to each well.

E. Incubate the wells at room temperature (18-25 °C) for 15 minutes.

F. Repeat washing as in section C above.

G. Add 100µl of ready-to-use TMB substrate to each well.

H. Incubate the wells at room temperature (18-25 °C) for 15 minutes.

I. Add 50µl of stop solution to each well. Tap gently to ensure uniform color distribution and read within 15 minutes.

J. To read the plate, ensure the base is free from moisture and no air bubbles are in the wells. Read the absorbance of the well contents at 450 nm on a suitable plate reader. On readers equipped with a dual wavelength facility set the reference filter to 600 - 650 nm.

9.3 PROCEDURAL NOTES

Do not allow the wells to dry between incubations.

Do not vary reagents and incubation temperatures above or below room temperature (18 - 25°C).

WASHING PROCEDURE

The washing procedure can be done manually with a multichannel pipette or on an automatic plate washer. Empty the wells, invert and tap dry on paper towel.

10.0 CALCULATION OF RESULTS

10.1 VALIDITY OF THE ASSAY

In order that the assay be valid, the following criteria must be fulfilled:

Adaltis supplies with each EIAgen C3d-CIC, positive and negative control samples which should be assayed with each run. The results of these quality control samples should fall within the limits indicated on the Certificate of Analysis.

- Results obtained for quality control sera must fall within acceptable ranges: please refer also to next section 10.2_QUALITY CONTROL.

10.2 QUALITY CONTROL

Good laboratory practice indicates that with each assay run, one or more quality control samples of known antibody level should be analyzed as though they were clinical samples

Should the results fall outwith this range repeat the assay using freshly prepared controls. Should the results continue to fall outside the specific range, and after equipment, adherence to the protocol and laboratory procedure have been verified, seek assistance from the supplier. Do not report patient results if the control results fall outwith the acceptable ranges.

10.3 OD CONVERSION

The optical densities (ODs) higher than 2.0 are out of the measurement range of some microplate readers. It is therefore necessary, for ODs higher than 2.0, to perform a reading at 405 nm (= wavelength of peak shoulder) in addition to 450 nm (peak wavelength) in addition to 450 nm (peak wavelength) and 620 nm (reference filter for the subtraction of interferences due to the plastic).

For microplate readers unable to read the plate at 3 wavelenghts at the same time, it is advisable to proceed as follows:

- Read the microplate at 450 nm and at 620 nm
- Read again the plate at 405 nm and 620 nm
- Find out the wells whose ODs at 450 nm are higher than 2.0
- Select the corresponding ODs read at 405 nm and multiply these values at 405 nm by the conversion factor 3.0 (where OD 450/Od 405 = 3.0), that is:

$$OD\ 450\ nm = OD\ 405\ nm \times 3.0$$

Warning: the conversion factor 3.0 is suggested only. For better accuracy, the user is advised to calculate the conversion factor specific for his own reader.

10.4 SEMI-QUANTITATIVE CALCULATION

Data Reduction – manual method

Plot the blank corrected optical densities (OD's) of the calibrators against the concentration values, using a linear y-axis (OD) and a logarithmic x-axis (concentration). Use the reagent blank as the zero calibrator. The concentration value of the patient samples can then be determined from this calibration curve.

Data reduction – automated method

Alternatively, if curve fitting software is available, the data may be processed using this. The curve is best described by a 2-point linear regression fit with linear axes. In order for the assay to be declared valid the following criteria should be met:

- Slope > 0.008
- Y-intercept < 0.600

11.0 EXPECTED VALUES

The assay cut-off was determined by combining data from a panel of 50 asymptomatic normal samples and a panel of other autoimmune positives. These are as follows:

µg/ml HAG	Negative	Equivocal	Positive
C3d CIC	<16	16 - 24	> 24

12.0 LIMITATIONS OF PROCEDURE

12.1 KNOWN INTERFERENCES

Grossly haemolysed, lipaemic or microbiologically contaminated samples should not be used. Samples with abnormally elevated levels of haemoglobin, bilirubin and especially EDTA may interfere with assay performance and accuracy.

A 'hook effect' may only be seen with very high samples which are above the assay range. No hook effect is seen up to 192µg/ml HAG. This is a sample specific effect.

12.2 CAUTIONS IN INTERPRETATIONS OF THE RESULTS

A negative result should not be used as a sole criterion to rule out immune dysfunctions but must be taken in relation to other clinical observations and diagnostic tests. While the precision of the EIAgen kit is sufficient to allow samples to be measured in single determinations, this is done at the clinical laboratory's discretion. It is advised that duplicate determinations should be used to enable identification of potential pipetting error or to allow for confirmation in the equivocal range.

It should be noted that C3d-containing CICs antibodies occur at low levels in other autoimmune and non-autoimmune conditions. Therefore all other clinical observations and diagnostic tests should be taken into account for clinical diagnosis.

13.0 PERFORMANCE CHARACTERISTICS

13.1 ANALYTICAL SPECIFICITY AND SENSITIVITY

A panel of 47 'normal' asymptomatic individuals was run on the kit. 90% of the normals gave negative results, and only 2% were positive. A panel of 50 autoimmune positive samples was tested.

	Type	Negative	Equivocal	Positive
Samples 1-27	RA	30%	18%	52%
Samples 28	ACA	100%	0%	0%
Samples 29-43	DNA	53%	13%	34%
Samples 44-50	Thyroid	57%	14%	29%
Normals 1-47	Normal	90%	8%	2%

The sensitivity of the assay was established by calculation of the mean plus two standard deviations of replicates of the zero calibrator which gave a value of 0.7 µg/ml

13.2 REPRODUCIBILITY

Intra- and inter- assay variation were checked using a number of samples.

Intra-assay variation	A	B	C
x	30.1	52.7	100.5
%cv	8.6	4.0	3.2
Inter-assay variation	A	B	C
x	14.2	52.8	78.5
%cv	2.5	3.7	11.1

14.0 AUTOMATION

Application protocols for the proper automation on the Adaltis microplate analyzers are available upon request at Adaltis directly.

15.0 SUGGESTIONS FOR TROUBLESHOOTING

Adherence to assay procedure and specifications, as well as a correct use of reagents and proper pipetting, may help to avoid the following kinds of errors .

ERROR	POSSIBLE CAUSES / SUGGESTIONS
OD very different (± 50%) from OD reported on QC	<ul style="list-style-type: none"> - incorrect dispensing volume of reagents (suggestion: check the correspondence between the volume dispensed by the pipette and the one required by the assay; re-calibrate again pipettes) - incorrect temperature or incorrect incubation time (suggestion: more care in the incubator maintenance; note down the beginning of the incubation) - error in washing or in spectrophotometer reading (suggestion: check operating or settings of respective instruments) - contamination of Substrate (suggestion: use only disposable and clean plastic containers)
Low reproducible results	<ul style="list-style-type: none"> - incorrect dispensing volume of reagents (suggestion: check the correspondence between the volume dispensed by the pipette and the one required by the assay; re-calibrate again pipettes) - incorrect temperature or incorrect incubation time (suggestion: more care in the incubator maintenance) - error in washing or in reading to spectrophotometer (suggestion: check operating or settings of respective instruments) - contamination of Substrate (suggestion: use only disposable and clean plastic containers) - pollution or degradation of reagents (suggestion: use appropriate tips, disposable and clean plastic containers for reagents and high quality distilled or equivalent water)
no colourimetric reaction after addition of substrate	<ul style="list-style-type: none"> - no reagent pipetted - strong contamination of conjugate or Substrate - errors in performing the assay procedure (e.g. accidental pipetting of reagents in a wrong sequence or from the wrong vial, etc.)
too low reaction (too low ODs)	<ul style="list-style-type: none"> - incorrect conjugate (e.g. not from original kit) - incubation time too short, incubation temperature too low
too high reaction (too high ODs)	<ul style="list-style-type: none"> - incorrect conjugate (e.g. not from original kit) - accidental contamination/degradation of conjugate - incubation time too long, incubation temperature too high - water quality for wash buffer insufficient (low grade of deionization) - insufficient washing (conjugates not properly removed)
unexplainable outliers	<ul style="list-style-type: none"> - contamination of pipettes, tips or containers - insufficient washing (conjugates not properly removed)
too high within-run CV%	<ul style="list-style-type: none"> - reagents and/or strips not pre-warmed to Room Temp. prior to use - plate washer is not washing correctly (suggestion: clean washer head)
too high between-run CV%	<ul style="list-style-type: none"> - incubation conditions not constant (time, temperature) - controls and samples not dispensed at the same time (with the same intervals) - (check pipetting order) - person-related variation

16.0 REFERENCES

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5. Pereira AB. Theofilopoulos AN. Dixon FJ. (1980) Detection and partial characterization of circulating immune complexes with solid-phase anti-C3. *J. Immunol.*, 125, 763-770.