CIC C1q ELISA | **for routine analysis**

Direct immunoenzymatic determination of CIC C1q in human serum or plasma

| IVD | ☣ | LOT See external label | 2°C ⟋ 8°C | ▽ Σ = 96 tests | **REF** DKO016 |

INTENDED USE

Eagle Biosciences CIC C1q ELISA Assay Kit is an Immunoenzymatic colorimetric method for quantitative determination of CIC C1q concentration in human serum or plasma.

CIC C1q ELISA Assay Kit is intended for research use only and not to be used in diagnostic procedures.

1. CLINICAL SIGNIFICANCE

The complement system is a biochemical cascade of the immune system that helps clear pathogens from an organism. It is derived from many small plasma proteins that work together to form the primary end result of cytolysis by disrupting the target cell's plasma membrane.

Activation of this system leads to cytolysis, chemotaxis, opsonization, immune clearance, and inflammation, as well as the marking of pathogens for phagocytosis. The complement system consists of more than 35 soluble and cell-bound proteins, 12 of which are directly involved in the complement pathways. The proteins account for 5% of the serum globulin fraction. The complement proteins are synthesized mainly by hepatocytes; however, significant amounts are also produced by monocytes, macrophages, and epithelial cells in the gastrointestinal and genitourinary tracts.

C1q is involved in the classical complement pathway. The classical pathway is triggered by activation of the C1-complex (which consists of one molecule C1q and two molecules C1r and C1s), either by C1q's binding to antibodies from classes M and G, complexed with antigens, or by its binding C1q to the surface of the pathogen.

The complement system might play a role in many diseases with an immune component, such as Barraquer-Simons Syndrome Alzheimer's disease, asthma, lupus erythematosus, various forms of arthritis, autoimmune heart disease and multiple sclerosis.Deficiencies of the terminal pathway predispose to both autoimmune disease and infections (particularly meningitis).

There are many tests for the determination of CIC, included the test of precipitation with PEG, radial immunodiffusion, and cellular tests like the test of Ray cell. Does not exist one procedure to determinate all types of immunocomplex; in commerce exist some test to determinate fragments of the complex (Es. C1q and C3d) that have an important diagnostic mean.

2. PRINCIPLE

CIC C1q ELISA Assay Kit is based on the binding of C1q-linked immunocomplexes to C1q adsorbed on microplate.

In the first step, the samples are added to the microplate adsorbed with C1q; during the following incubation, C1q-fixing circulating immune complexes (CIC) bind to the C1q immobilized on the microplate. The microplate is washed for remove the unbound serum proteins.

In the second step, the anti-human IgG conjugated with horseradish peroxidase (HRP) is added; it binds to the immunocomplex fixed on the microplate. The washing step removes the unbound conjugate.

In the third step, the TMB Substrate is added, and this reacts with the conjugate fixed on the microplate, developing a colorimetric reaction.

The quantity or CIC IgG complex is proportional to the colour intensity read at 450 nm wavelengths.

The immunocomplex concentration in the sample is calculated through a calibration cuve.

Heat aggregate human gamma globulin per mL (μgEq/mL) is the unit of measure of the results.

3. REAGENTS, MATERIALS AND INSTRUMENTATION

3.1. Reagents and materials supplied in the kit

1. CIC C1q Calibrators (3 vials, 1.5 mL each)

CAL0	**REF** DCE002/1606-0
CAL1	**REF** DCE002/1607-0
CAL2	**REF** DCE002/1608-0

2. Controls (2 vials, 1.5 mL each, ready to use)
74 mM Phosphate buffer, pH 7.4, 1 g/L BSA

Negative Control	**REF** DCE045/1601-0
Positive Control	**REF** DCE045/1602-0

3. Incubation Buffer (1 vial, 50 mL)
74 mM Phosphate buffer, pH 7.4 **REF** DCE008-0

4. Conjugate (1 vial, 0.5 mL)
Anti human IgG conjugated with horseradish peroxidase (HRP) **REF** DCE002/1602-0

5. Conjugate Buffer (1 vial, 20 mL)
74 mM Phosphate buffer, pH 7.4 **REF** DCE009-0

6. Coated Microplate (1 breakable microplate)
Microplate coated with C1q **REF** DCE002/1603-0

7. 10X Conc. Wash Solution (2 vials, 50 mL each)
NaCl 160 g/L; tween-20 10 g/L - 0.2M Phosphate buffer, pH 7.4 **REF** DCE054-0

8. TMB Substrate (1 vial, 15 mL)
H_2O_2-TMB 0.26 g/L *(avoid any skin contact)*
  REF DCE004-0

9. Stop Solution (1 vial, 15 mL)
Sulphuric acid 0.15 mol/L *(avoid any skin contact)*
  REF DCE005-0

3.2. Reagents necessary not supplied

Distilled water.

3.3. Auxiliary materials and instrumentation

Automatic dispenser.

Microplates reader (450 nm)

Note

Store all reagents at 2-8°C in the dark.

Open the bag of reagent 6 (Coated Microplate) only when it is at room temperature and close it immediately after use.

4. WARNINGS

- This CIC C1q ELISA Assay Kit is intended for in vitro use by professional persons only. Not for internal or external use in Humans or Animals.
- Use appropriate personal protective equipment while working with the reagents provided.
- Follow Good Laboratory Practice (GLP) for handling blood products.
- All human source material used in the preparation of the reagents has been tested and found negative for antibody to HIV 1&2, HbsAg, and HCV. No test method however can offer complete assurance that HIV, HBV, HCV or other infectious agents are absent. Therefore, Calibrators and Controls should be handled in the same manner as potentially infectious material.
- Material of animal origin used in the preparation of the kit has been obtained from animals certified as healthy and the bovine protein has been obtained from countries not infected by BSE, but these materials should be handled as potentially infectious.
- Some reagents of this CIC C1q ELISA Assay Kit contain small amounts of Proclin 300^R as preservatives. Avoid the contact with skin or mucosa.
- The TMB Substrate contains an irritant, which may be harmful if inhaled, ingested or absorbed through the skin. To prevent injury, avoid inhalation, ingestion or contact with skin and eyes.
- The Stop Solution consists of a diluted sulphuric acid solution. Sulphuric acid is poisonous and corrosive and can be toxic if ingested. To prevent chemical burns, avoid contact with skin and eyes.
- Avoid the exposure of reagent TMB/H_2O_2 to directed sunlight, metals or oxidants. Do not freeze the solution.

5. PRECAUTIONS

- Please adhere strictly to the sequence of pipetting steps provided in this protocol. The performance data represented here were obtained using specific reagents listed in this Instruction For Use.
- All reagents of the CIC C1q ELISA Assay Kit should be stored refrigerated at 2-8°C in their original container. Any exceptions are clearly indicated. The reagents are stable until the expiry date when stored and handled as indicated.
- Allow all CIC C1q ELISA Assay Kit components and specimens to reach room temperature (22-28°C) and mix well prior to use.
- Do not interchange CIC C1q ELISA Assay Kit components from different lots. The expiry date printed on box and vials labels must be observed. Do not use any kit component beyond their expiry date.
- **WARNING: the conjugate reagent is designed to ensure maximum dose sensitivity and may be contaminated by external agents if not used properly;** therefore, it is recommended to use disposable consumables (tips, bottles, trays, etc.). For divided doses, take the exact amount of conjugate needed and do not re-introduce any waste product into the original bottle. In addition, **for doses dispensed with the aid of automatic and semi-automatic devices,** before using the conjugate, it is advisable to clean the fluid handling system, ensuring that the procedures of washing, deproteinization and decontamination are effective in avoiding contamination of the conjugate; this procedure is highly recommended when the kit is processed using analyzers which are not equipped with disposable tips.

For this purpose, Diametra supplies a separate decontamination reagent for cleaning needles.

- If you use automated equipment, the user has the responsibility to make sure that the kit has been appropriately tested.
- The incomplete or inaccurate liquid removal from the wells could influence the assay precision and/or increase the background.
- It is important that the time of reaction in each well is held constant for reproducible results. Pipetting of samples should not extend beyond ten minutes to avoid assay drift. If more than 10 minutes are needed, follow the same order of dispensation. If more than one plate is used, it is recommended to repeat the dose response curve in each plate
- Addition of the TMB Substrate solution initiates a kinetic reaction, which is terminated by the addition of the Stop Solution. Therefore, the TMB Substrate and the Stop Solution should be added in the same sequence to eliminate any time deviation during the reaction.
- Observe the guidelines for performing quality control in medical laboratories by assaying controls and/or pooled sera.
- Maximum precision is required for reconstitution and dispensation of the reagents.
- Samples microbiologically contaminated, highly lipemeic or haemolysed should not be used in the assay.
- Plate readers measure vertically. Do not touch the bottom of the wells.

6. PROCEDURE

6.1. Calibrators preparation

The Calibrators are ready to use and have the following concentrations:

	C_0	C_1	C_2
µgEq/mL	0	16	64

Allow the Calibrators to reach room temperature (22-28°C) before use. Mix gently. Once opened, the Calibrators are stable 6 months at 2-8°C.

6.2. Preparation of Diluted Conjugate

Dilute the Conjugate (reagent 4) 1:100 with Conjugate buffer (reagent 5). The exact quantity is proportional to the number of the assays.

Mix well and avoid foaming. Stable for 3 hours at room temperature (22÷28°C).

6.3. Preparation of Wash Solution

Dilute the contents of each vial of the buffered wash solution concentrate (10X) with distilled water to a final volume of 500 mL prior to use. For smaller volumes respect the 1:10 dilution ratio. The diluted wash solution is stable for 30 days at 2-8°C.

In concentrated wash solution it is possible to observe the presence of crystals. In this case mix at room temperature until complete dissolution of crystals is observed. For greater accuracy dilute the whole bottle of concentrated wash solution to 500 mL taking care also to transfer the crystals completely, then mix until the crystals are completely dissolved.

6.4. Preparation of the Sample

The CIC assay can be performed in human serum or plasma. Samples that are not immediately processed

should be stored at -20°C. Samples should not be thawed more than once.
Prepare the samples by pipetting in a test tube:

Sample	10 µL
Incubation Buffer (reagent 3)	**500 µL**

Mix gently. Avoid using vortex.
The Controls are ready to use.

6.5. Procedure

- **Allow all reagents to reach room temperature (22-28°C) for at least 30 minutes.**
- Unused coated microwell strips should be released securely in the foil pouch containing desiccant and stored at 2-8°C.
- To avoid potential microbial and/or chemical contamination, unused reagents should never be transferred into the original vials.
- As it is necessary to perform the determination in duplicate in order to improve accuracy of the test results, prepare two wells for each point of the calibration curve (C_0-C_2), two for each Control, two for each sample, one for Blank.

Reagents	Calibrator	Sample/ Controls	Blank
Calibrator C_0-C_2	100 µL		
Controls		100 µL	
Diluted Sample		100 µL	
Incubate 30 minutes at 37°C. Remove the contents from each well and wash the wells 3 times with 300 µL diluted wash solution .			
Diluted Conjugate	100 µL	100 µL	
Incubate 30 minutes at 37°C. Remove the contents from each well, wash the wells 3 times with 300 µL diluted wash solution.			
TMB Substrate	100 µL	100 µL	100 µL
Incubate 15 minutes in the dark at room temperature (22÷28°C).			
Stop Solution	100 µL	100 µL	100 µL
Shake the microplate gently. Read Absorbance (E) at 450 nm against Blank within 5 minutes.			

7. QUALITY CONTROL

Each laboratory should assay controls at normal, high and low levels range of CIC C1q for monitoring assay performance. These controls should be treated as unknowns and values determined in every test procedure performed. Quality control charts should be maintained to follow the performance of the supplied reagents. Pertinent statistical methods should be employed to ascertain trends. The individual laboratory should set acceptable assay performance limits. In addition, maximum absorbance should be consistent with past experience. Significant deviation from established performance can indicate unnoticed change in experimental conditions or degradation of kit reagents. Fresh reagents should be used to determine the reason for the variations.

8. RESULTS

8.1. Mean Absorbance
Calculate the mean of the absorbance (Em) for each point of the calibration curve and of each sample.

8.2. Calibration curve
Plot the mean value of absorbance of each Calibrator (Em) against concentration. Draw the best-fit curve through the plotted points.

8.3. Calculation of Results
Interpolate the values of the samples on the calibration curve to obtain the corresponding values of the concentrations expressed in µgEq/mL.

9. REFERENCE VALUES

	µgEq/mL of aggregates IgG
Negative Sample	<16
Uncertain Sample	between 16 and 18
Positive Sample	>18

Please pay attention to the fact that the determination of a range of expected values for a "normal" population in a given method is dependent on many factors, such as specificity and sensitivity of the method used and type of population under investigation. Therefore each laboratory should consider the range given by the Manufacurer as a general indication and produce their own range of expected values based on the indigenous population where the laboratory works.

10. PERFORMANCE AND CHARACTERISTICS

10.1. Precision
10.1.1. Intra Assay Variation
Within run variation was determined by replicate the measurement (16x) of two different control sera in one assay. The within assay variability is ≤ 5.3%.
10.1.2. Inter Assay Variation
Between run variations was determined by replicate the measurements of two different control sera in 2 different lots. The between assay variability is ≤ 6.0%.

10.2. Recovery
The recovery of 12.5 – 25 – 50 – 100 µgEq/mL IgG aggregates added to a sample gave values between 94.3% and 105.7% with reference to the original concentrations.

10.3. Detection limit
The lowest detectable concentration of CIC C1q that can be distinguished from the zero Calibrator is 1.0 µgEq/mL at the 99% confidence limit.

10.4. Specificity and sensitivity
10.4.1. Specificity and sensitivity
92 serum specimens collected from normal, asymptomatic subjects were tested with CIC C1q ELISA DiaMetra. The specificity of the assay was 96%.
125 serum specimen collected from subjects with systemic lupus erymatosus (SLE), rheumatoid arthritis (RA) or other disorders was tested with CIC C1q. The overall clinical sensitivity was 92%.

10.4.2. Specificity and sensitivity vs commercial reference method
Specimen obtained from 209 subjects with SLE, RA, or other disorders were tested using the Diametra CIC C1q kit and QUIDEL EIA kit. The obtained results are shown in the table below:

DIAMETRA kit	-	+	-	+
QUIDEL kit	-	+	+	-
RA Subjects	20	12	4	4
SLE Subjects	38	25	12	6
Others	0	85	2	1

From the 209 tested samples the following sensitivity and specificity are obtained:

	RA	SLE	Others	RA+ SLE	RA+ SLE+ Others
Sensitivity	75,0%	67,6%	97,7%	69,8%	87,1%
Specificity	83,3%	86,4%	--	85,3%	84,1%
Agreement	-	-	-	78,5%	86,1%

10.4.3. Comparative data

Circulating Immunocomplexes (CIC) collected from 160 patient with systemic lupus erythematosus (SLE), rheumatoid arthritis (RA), or other disorders subjects, and 95 form normal, asymptomatic subjects were measured.

The overall agreement between the two test methods was 87%.

The average CIC concentration of healthy donors was 2.1 µgEq/mL (S.D. = 1.6).

11. WASTE MANAGEMENT

Reagents must be disposed off in accordance with local regulations.

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ERROR POSSIBLE CAUSES / SUGGESTIONS

No colorimetric reaction
- no conjugate pipetted reaction after addition
- contamination of conjugates and/or of 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)
- incorrect conjugate (e.g. not from original kit)
- incubation time too short, incubation temperature too low

 Too high reaction (too high ODs)
- incorrect conjugate (e.g. not from original kit)
- 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
- contamination of pipettes, tips or containers
insufficient washing (conjugates not properly removed) too high within run
- reagents and/or strips not pre-warmed to CV% Room Temperature prior to use
- plate washer is not washing correctly (suggestion: clean washer head)
too high between-run - incubation conditions not constant (time, CV % temperature)
- controls and samples not dispensed at the same time (with the same intervals) (check pipetting order)
- person-related variation