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Instructions for Use

CIC-C3d ELISA

Enzyme immunoassay for the quantitative determination of CIC-C3d in human serum or plasma.

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I B L I N T E R N A T I O N A L G M B H
Flughafenstrasse 52a
D-22335 Hamburg, Germany
Phone: +49 (0)40-53 28 91-0
Fax: +49 (0)40-53 28 91-11
IBL@IBL-International.com
www.IBL-International.com

1. INTENDED USE

Immunoenzymatic colorimetric method for quantitative determination of Circulating Immune Complex C3d (CIC C3d) concentration in human serum or plasma. CIC C3d ELISA kit is intended for laboratory use only.

2. SUMMARY AND EXPLANATION

The importance of the immunocomplex (CIC) and their relation with several diseases have been object of investigations for many years.
The enstablishment of immunocomplex is a normal protecting process of the immune system. The circulating immunocomplex are removed from the circulation by means of various cellular, biochemical and enzymatic processes.
Key of elimination of many CIC is the activation of the classic way of the complement.
In some diseases, of difficult understanding, the immunocomplex can begin the damaging of tissue and organs. In this case the activation of the complement can lead to the anafilotoxine production, stimulation of leukocyte and activation of macrophage and other cells.
In some cases of glomerulonephritis, in which the immunocomplex fix to the cellular membranes, it has the destruction of the tissue.
Circulating immuno-complexes (CIC) are present in many individuals affections from systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA), especially in those affections from vasculitis complications. 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 some test to determinate fragments of the complex are available (Es. C1q and C3d), that have an important diagnostic meaning.

3. TEST PRINCIPLE

C3d-fixing circulating immune complexes (CIC) are first blocked by the anti-C3d immobilized on the microplate. During this phase, the immunocomplex bind the anti-C3d coated on the microplate. The microplate is washed for remove the unbound serum protein. In the second phase a coniugate anti-human IgGperoxidase is added, which will bind to the immunocomplex fixed on the microplate. The washing solution removes the unbound conjugate. In the third phase, TMB-substrate is added, and this reacts with the conjugate fixed on the microplate. The quantity of CIC IgG complex is proportional to the colour intensity read at 450 nm wavelengths. The immunocomplex concentration in the sample is calculated based on a standard curve. Heat aggregate human gamma globulin per mL (µgEq/mL) is the unit of measure of the results.

4. REAGENTS, MATERIALS AND INSTRUMENTATION

4.1. Reagents and materials supplied in the kit

1. **MTP** Microtiter Plate (1 breakable microplate) Anti C3d antibodies coated on microplate
2. **ENZCONJ** **CONC** Enzyme Conjugate, Concentrate (100x), (1 vial, 0.5 mL) Anti human IgG antibodies conjugated with horseradish peroxidase (HRP)
3. **ENZCONJDIL** Conjugate Buffer (1 vial, 20 mL) Phosphate buffer 74 mM pH 7.4; BSA 1 g/L
4. **CAL A** **CAL B** **CAL C** Standard A-C (3 vials, 1.5 mL each)
5. **CONTROL -** / **CONTROL +** Controls Negative Control / Positive Control (2 vials, 1.5 mL each)
6. **TMB SUBS** Substrate Solution (1 vial, 15 mL) H_2O_2-TMB (0.26 g/L) *(avoid any skin contact)*
7. **TMB STOP** Stop Solution (1 vial, 15 mL) Sulphuric Acid 0.15 mol/L *(avoid any skin contact)*
8. **WASHBUF** **CONC** Wash Buffer, Concentrate (10x), (2 vials, 50 mL each) Phosphate buffer 0.2M, pH 7.4
9. **SAMPLEDIL** Sample Diluent (1 vial, 50 mL) Phosphate buffer 74 mM pH 7.4; BSA 1 g/L

4.2. Reagents necessary not supplied

Distilled water.

4.3. Auxiliary materials and instrumentation

Automatic dispenser.
Microplates reader (450 nm, 620-630 nm)

Note: Store all reagents at 2÷8°C in the dark.
Open the bag of the Microtiterplate only when it is at room temperature and close it immediately after use; once opened, the microplate is stable until the expiry date of the kit.

5. WARNINGS

- This 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 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.

6. 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 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 kit components and specimens to reach room temperature (22-28°C) and mix well prior to use.
- Do not interchange 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 Enzyme Conjugate 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.
- 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. To improve the performance of the kit on automatic systems is recommended to increase the number of washes.
- 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.

7. PROCEDURE

7.1. Preparation of Calibrators

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

	CAL A	CAL B	CAL C
µgEq/mL	0	16	64

Once opened the Calibrators are stable 6 months at 2-8°C.

7.2. Preparation of Diluted Conjugate

Dilute the concentrated Conjugate ENZCONJ 1:100 with Conjugate buffer ENZCONJDIL. Mix well and avoid foaming. Stable for 3 hours at room temperature (22-28°C).

7.3. Preparation of Wash Solution

Dilute the content of the vial "10X Conc. Wash Solution" 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, for greater accuracy dilute the whole bottle of concentrated wash solution to 500 mL taking care also to transfer crystals, then mix until crystals are completely dissolved.

7.4. Preparation of the Sample

The CIC assay can be performed in human serum or plasma. Samples, which are not immediately processed (within 24 h), should be stored at -20°C. Samples should not be thawed more than once.
Pipette in a test tube:

Serum	10 µL
Sample Diluent SAMPLEDIL	500 µL

Mix gently. Avoid vortex use.

7.5. Procedure

- **Allow all reagents to reach room temperature (22-28°C) for at least 30 minutes.** At the end of the assay, store immediately the reagents at 2-8°C: avoid long exposure to room temperature.
- 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 (CAL A – CAL B), two for each Control, two for each sample, one for Blank.

Reagents	Calibrator	Sample/ Controls	Blank
Calibrator CAL A – CAL C	**100 µL**		
Controls		**100 µL**	
Diluted Sample		**100 µL**	
Incubate 30 minutes at 37°C. Remove the contents from each well, wash the wells three times with 300 µL diluted wash solution. **Important note**: during each washing step, gently shake the plate for 5 seconds and remove excess solution by tapping the inverted plate on an absorbent paper towel. **Automatic washer**: if you use automated equipment, wash the wells at least 5 times.			
Diluted Conjugate	**100 µL**	**100 µL**	
Incubate 30 minutes at 37°C. Remove the contents from each well, wash the wells three times with 300 µL diluted wash solution. **Washing**: follow the same indications of the previous point.			
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 the absorbance (OD) at 450 nm against a reference wavelength of 620-630 nm or against Blank within 5 minutes.			

8. QUALITY CONTROL

Each laboratory should assay controls at normal, high and low levels range of CIC C3d 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. Other parameters that should be monitored include the 80, 50 and 20% intercepts of the calibration curve for run-to-run reproducibility. 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.

9. RESULTS

9.1. Mean Absorbance

Calculate the mean of the absorbance (OD_{mean}) for each point of the calibration curve and of each sample.

9.2. Calibration curve

Plot the mean value of absorbance (OD_{mean}) of the Calibrators (CAL A – CAL C) against concentration. Draw the best-fit curve through the plotted points. (eg: Four Parameter Logistic).

9.3. 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.

10. 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.

11. PERFORMANCE CHARACTERISTICS

11.1. Precision

Intra-Assay Variation
Within run variation was determined by replicate measurements (16x) of two different control sera in one assay. The within assay variability is 6.1 %.

Inter Assay Variation
Between run variations was determined by replicate measurements of three different control sera in 2 different lots. The between assay variability is 13.9 %.

11.2. Accuracy

The recovery of 12.5 – 25 – 50 µgEq/mL CIC C3d added to “serum-free” sample gave an average value (±SD) of 99.84 % ± 5.07 % with reference to the original concentrations.

11.3. Sensitivity

The lowest detectable concentration of CIC C3d that can be distinguished from the zero standard is 0.6 µgEq/mL at the 95 % confidence limit.

12. WASTE MANAGEMENT

Reagents must be disposed in accordance with local regulations.

13. PRODUCT LITERATURE REFERENCES

1. Triolo G., et al J.Clin.Lab.Immunol 13, 35-39 (1984)
2. Rong-jia Xu et al J.Immunol.Met. 135, 225-231(1990)
3. Menzel J.E., et al J.Immunol. Met. 138, 16 (1991)
4. Muso E, et al Nippon Jinzo Gakkai Shi.36(4):345-54 (1994)
5. Yoshinoya S, et al J Clin Lab Immunol. 38(4):161-73 (1992)

14. 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)

To 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)

To 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

Symbols / Symbole / Symbôles / Símbolos / Símbolos / Σύμβολα

REF	Cat.-No.: / Kat.-Nr.: / No.- Cat.: / Cat.-No.: / N.º Cat.: / N.–Cat.: / Αριθμός-Κατ.:
LOT	Lot-No.: / Chargen-Bez.: / No. Lot: / Lot-No.: / Lote N.º: / Lotto n.: / Αριθμός -Παραγωγή:
	Use by: / Verwendbar bis: / Utiliser à: / Usado por: / Usar até: / Da utilizzare entro: / Χρησιμοποιείται από:
	No. of Tests: / Kitgröße: / Nb. de Tests: / No. de Determ.: / N.º de Testes: / Quantità dei tests: / Αριθμός εξετάσεων:
CONC	Concentrate / Konzentrat / Concentré / Concentrar / Concentrado / Concentrato / Συμπύκνωμα
LYO	Lyophilized / Lyophilisat / Lyophilisé / Liofilizado / Liofilizado / Liofilizzato / Λυοφιλιασμένο
IVD	In Vitro Diagnostic Medical Device. / In-vitro-Diagnostikum. / Appareil Médical pour Diagnostics In Vitro. / Dispositivo Médico para Diagnóstico In Vitro. / Equipamento Médico de Diagnóstico In Vitro. / Dispositivo Medico Diagnostico In vitro. / Ιατρική συσκευή για In-Vitro Διάγνωση.
	Evaluation kit. / Nur für Leistungsbewertungszwecke. / Kit pour évaluation. / Juego de Reactivos para Evaluació. / Kit de avaliação. / Kit di evaluazione. / Κιτ Αξιολόγησης.
	Read instructions before use. / Arbeitsanleitung lesen. / Lire la fiche technique avant emploi. / Lea las instrucciones antes de usar. / Ler as instruções antes de usar. / Leggere le istruzioni prima dell'uso. / Διαβάστε τις οδηγίες πριν την χρήση.
	Keep away from heat or direct sun light. / Vor Hitze und direkter Sonneneinstrahlung schützen. / Garder à l'abri de la chaleur et de toute exposition lumineuse. / Manténgase alejado del calor o la luz solar directa. / Manter longe do calor ou luz solar directa. / Non esporre ai raggi solari. / Να φυλάσσεται μακριά από θερμότητα και άμεση επαφή με το φως του ηλίου.
	Store at: / Lagern bei: / Stocker à: / Almacene a: / Armazenar a: / Conservare a: / Αποθήκευση στους:
	Manufacturer: / Hersteller: / Fabricant: / Productor: / Fabricante: / Fabbricante: / Παραγωγός:
	Caution! / Vorsicht! / Attention! / ¡Precaución! / Cuidado! / Attenzione! / Προσοχή!

Symbols of the kit components see MATERIALS SUPPLIED.
Die Symbole der Komponenten sind im Kapitel KOMPONENTEN DES KITS beschrieben.
Voir MATERIEL FOURNI pour les symbôles des composants du kit.
Símbolos de los componentes del juego de reactivos, vea MATERIALES SUMINISTRADOS.
Para símbolos dos componentes do kit ver MATERIAIS FORNECIDOS.
Per i simboli dei componenti del kit si veda COMPONENTI DEL KIT.
Για τα σύμβολα των συστατικών του κιτ συμβουλευτείτε το ΠΑΡΕΧΟΜΕΝΑ ΥΛΙΚΑ.

COMPLAINTS: Complaints may be submitted initially written or vocal. Subsequently they need to be filed including the test performance and results in writing in case of analytical reasons.

WARRANTY: The product is warranted to be free from material defects within the specific shelf life and to comply with product specifications delivered with the product. The product must be used according to the Intended use, all instructions given in the instructions for use and within the product specific shelf life. Any modification of the test procedure or exchange or mixing of components of different lots could negatively affect the results. These cases invalidate any claim for replacement.

LIMITATION OF LIABILITY: IN ALL CIRCUMSTANCES THE EXTENT OF MANUFACTURER'S LIABILITY IS LIMITED TO THE PURCHASE PRICE OF THE KIT(S) IN QUESTION. IN NO EVENT SHALL MANUFACTURER BE LIABLE FOR ANY INCIDENTAL OR CONSEQUENTIAL DAMAGES, INCLUDING DAMAGES FOR LOST PROFITS, LOST SALES, INJURY TO PERSON OR PROPERTY OR ANY OTHER INCIDENTAL OR CONSEQUENTIAL LOSS.


IBL International GmbH
Flughafenstr. 52A, 22335 Hamburg, Germany

Tel.: + 49 (0) 40 532891 -0 Fax: -11
E-MAIL: IBL@IBL-International.com
WEB: http://www.IBL-International.com