

ab178665

CIC-C3d ELISA Kit

Instructions for Use

An immunoenzymatic assay for the quantitative measurement of CIC-C3d in Human serum and plasma.

This product is for research use only and is not intended for diagnostic use.

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1. BACKGROUND

Abcam's CIC-C3d *in vitro* ELISA (Enzyme-Linked Immunosorbent Assay) kit is designed for the accurate quantitative measurement of CIC-C3d in Human serum and plasma.

A 96-well plate has been pre-coated with anti-C3d antibodies. Standards, controls and test samples are added to the wells and incubated. Immune complexes (CIC) in the samples and standards bind to the pre-coated antibody. Following washing, a horseradish peroxidase (HRP) labelled anti-Human IgG conjugate is added to the wells, which binds to the immobilized CIC-C3d. After incubation, the wells are washed to remove unbound material and TMB substrate is then added which is catalyzed by HRP to produce blue coloration. The reaction is terminated by addition of Stop Solution which stops the color development and produces a color change from blue to yellow. The intensity of signal is directly proportional to the amount of CIC-C3d in the sample and the intensity is measured at 450 nm.

Heat aggregate Human gamma globulin per mL ($\mu\text{g Eq/mL}$) is the unit of measure given in the results.

The importance of the immuno-complex (CIC) and their relation with several diseases have been object of investigations for many years.

The establishment of immuno-complex is a normal protecting process of the immune system. The circulating immuno-complex 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 complex diseases, the immuno-complex 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 glomerulonephritises, in which the immune-complex bind to the cellular membranes, it destroys the tissue.

Circulating immuno-complexes (CIC) are present in many individual affections from systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA), especially in those affected from vasculitides complications.

There are many tests for the determination of CIC, including the test of precipitation with PEG, radial immunodiffusion, and cellular tests like the test of Ray cell.

There is currently no procedure to determinate all types of immune-complex; in commerce some test to determinate fragments of the complex are available (es. C1q and C3d), that have an important diagnostic meaning.

2. ASSAY SUMMARY

Primary capture antibody



Prepare all reagents, samples and standards as instructed.

Sample



Add samples, standards and controls to wells used. Incubate at 37°C.

HRP conjugated antibody



Add prepared HRP-conjugate mixture to each well. Incubate at 37°C.

Substrate **Colored product**



After washing, add TMB substrate solution to each well. Incubate at room temperature. Add Stop Solution to each well. Read immediately.

3. PRECAUTIONS

Please read these instructions carefully prior to beginning the assay.

All kit components have been formulated and quality control tested to function successfully as a kit. Modifications to the kit components or procedures may result in loss of performance.

4. STORAGE AND STABILITY

Store kit at 2-8°C immediately upon receipt.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in section 9. Reagent Preparation.

5. MATERIALS SUPPLIED

Item	Amount	Storage Condition (Before Preparation)
Anti-CIC-C3d Coated Microplate (12 x 8 wells)	96 Wells	2-8°C
Stop Solution	15 mL	2-8°C
CIC-C3d Anti-IgG HRP Conjugate (Concentrated)	0.5 mL	2-8°C
TMB Substrate Solution	15 mL	2-8°C
10X Washing Solution	2 x 50 mL	2-8°C
Incubation buffer	50 mL	2-8°C
CIC-C3d Positive Control	1.5 mL	2-8°C
CIC-C3d Negative Control	1.5 mL	2-8°C
CIC-C3d Standard 0 – 0 µg Eq/mL	1.5 mL	2-8°C
CIC-C3d Standard 1 – 16 µg Eq/mL	1.5 mL	2-8°C
CIC-C3d Standard 2 – 64 µg Eq/mL	1.5 mL	2-8°C
Conjugate Buffer	20 mL	2-8°C
Strip Holder	1 unit	2-8°C
Cover Foil	1 unit	2-8°C

6. MATERIALS REQUIRED, NOT SUPPLIED

These materials are not included in the kit, but will be required to successfully utilize this assay:

- Microplate reader capable of measuring absorbance at 450 nm or 620 – 630 nm
- Multi- and single-channel pipettes to deliver volumes between 10 and 1,000 µL
- Optional: Automatic plate washer for rinsing wells.
- Rotating mixer
- Deionised or (freshly) distilled water.
- Disposable tubes
- Timer

7. LIMITATIONS

- ELISA kit intended for research use only. Not for use in diagnostic procedures
- All components of Human origin used for the production of these reagents have been tested for anti-HIV antibodies, anti-HCV antibodies and HBsAg and have been found to be non-reactive. Nevertheless, all materials should still be regarded and handled as potentially infectious
- Use only clean pipette tips, dispensers, and lab ware
- Do not interchange screw caps of reagent vials to avoid cross-contamination
- Close reagent vials tightly immediately after use to avoid evaporation and microbial contamination
- After first opening and subsequent storage check conjugate and control vials for microbial contamination prior to further use
- To avoid cross-contamination and falsely elevated results pipette patient samples and dispense conjugate, without splashing, accurately to the bottom of wells

8. TECHNICAL HINTS

- Avoid foaming or bubbles when mixing or reconstituting components
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions
- Ensure plates are properly sealed or covered during incubation steps
- Complete removal of all solutions and buffers during wash steps is necessary for accurate measurement readings
- 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
- 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
- The incomplete or inaccurate liquid removal from the wells could influence the assay precision and/or increase the background. To improve performance of the kit on automatic systems it is recommended to increase the number of washes
- **This kit is sold based on number of tests. A ‘test’ simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions**

9. REAGENT PREPARATION

Equilibrate all reagents, samples and controls to room temperature (18-25°C) prior to use. At the end of the assay, store immediately the reagents at 2-8°C; avoid long exposure to room temperature.

9.1 **1X Washing Solution**

Prepare 1X Washing Solution by diluting 10X Washing Solution with deionized water. To make 200 mL 1X Washing Solution combine 20 mL 10X Washing Solution with 180mL deionized water. Mix thoroughly and gently.

9.2 **1X CIC-C3d Anti-IgG HRP Conjugate**

Dilute the CIC-C3d Anti-IgG HRP Conjugate (Concentrated) solution 1/100 with Conjugate buffer. The quantity of diluted Conjugate is proportional at the number of tests. Mix well and avoid foaming. The 1X CIC-C3d Conjugate should be prepared immediately before use. If required however, the 1X CIC-C3d Conjugate is stable for 3 hours at room temperature.

- All other solutions are supplied ready to use

10. SAMPLE COLLECTION AND STORAGE

- Use Human serum or plasma samples with this assay. If the assay is performed within 24 Hours of sample collection, the specimen should be kept at 2-8°C; otherwise it should be aliquoted and stored deep-frozen (-20 to -70°C). If samples are stored frozen, mix thawed samples well before testing.
- Samples must be diluted in the following way: Add 10 µL sample to 500 µL incubation buffer. Mix gently. Avoid vortex use.
Avoid repeated freezing and thawing.

11. PLATE PREPARATION

- The 96 well plate strips included with this kit are supplied ready to use. It is not necessary to rinse the plate prior to adding reagents
- Unused well strips should be returned to the plate packet and stored at 4°C.
- For each assay performed, a minimum of 1 well must be used as a blank, omitting sample and conjugate from well addition
- For statistical reasons, we recommend each standard and sample should be assayed with a minimum of two replicates (duplicates)

12. ASSAY PROCEDURE

- **Equilibrate all materials and prepared reagents to room temperature prior to use.**
- **Please read the test protocol carefully before performing the assay. Result reliability depends on strict adherence to the test protocol as described.**
- **If performing the test on ELISA automatic systems we recommend increasing the washing steps from three to five and the volume of washing solution from 300 μ L to 350 μ L to avoid washing effects.**
- **Assay all standards, controls and samples in duplicate.**
- **All controls (CIC-C3d positive control, CIC-C3d negative control) must be included with each assay performed to determine the test results.**

- 12.1. Prepare all reagents, working standards, and samples as directed in the previous sections.
- 12.2. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, reseal and return to 4°C storage.
- 12.3. Add 100 μ L standards, control and samples into their respective wells. Leave one well for substrate blank.
- 12.4. Cover wells with the foil supplied in the kit and incubate for 30 minute at 37 °C.
- 12.5. Remove the foil, aspirate the contents of the wells and wash each well three times with 300 μ L of 1X Washing Solution. Avoid spill over into neighboring wells. Invert the plate and blot it against clean paper towels to remove excess liquid.
Important note: During each washing step, gently shake the plate for 5 seconds. Complete removal of liquid at each step is essential for good assay performance.
- 12.6. Add 100 μ L diluted CIC-C3d Anti-IgG HRP conjugate to each well expect for the blank well.

ASSAY PROCEDURE

- 12.7. Cover wells with the foil supplied in the kit and incubate for 30 minute at 37 °C.
- 12.8. Repeat step 12.5
- 12.9. Add 100 μ L TMB Substrate Solution into all wells.
- 12.10. Incubate for exactly 15 minutes at room temperature in the dark.
- 12.11. Add 100 μ L Stop Solution into all wells in the same order and at the same rate as for the TMB Substrate Solution. Shake the microplate gently. Any blue color developed during the incubation turns into yellow.
- 12.12. Measure the absorbance of the sample at 450 nm against a reference wavelength of 620-630 nm or against blank within 5 minutes.

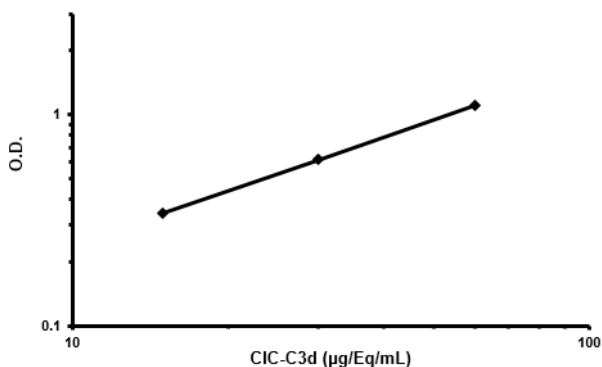
13. CALCULATIONS

Calculate the mean background subtracted absorbance for each point of the standard curve and each sample. Plot the mean value of absorbance of the standards against concentration. Draw the best-fit curve through the plotted points. (e. g.: Four Parameter Logistic).

Interpolate the values of the samples on the standard curve to obtain the corresponding values of the concentrations expressed in $\mu\text{g}/\text{Eq}/\text{mL}$.

14. TYPICAL DATA

TYPICAL STANDARD CURVE – Data provided for demonstration purposes only. A new standard curve must be generated for each assay performed.



Conc. (µg/Eq/mL)	O.D
0	0.04
15	0.34
30	0.61
60	1.11

15. TYPICAL SAMPLE VALUES

REFERENCE VALUES-

	µg/Eq/mL of aggregates IgG
Negative	< 16
Uncertain	16-18
Positive	>18

16. ASSAY ANALYTICAL SPECS

PRECISION -

	Intra-Assay	Inter-Assay
n=	16	
%CV	6.1	13.9

RECOVERY –

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

SENSITIVITY -

The lowest detectable concentration of CIC C3d that can be distinguished from standard 0 is 0.60 µg Eq/mL at the 95 % confidence limit.

17. TROUBLESHOOTING

Problem	Cause	Solution
Low signal	Incubation time too short	Try overnight incubation at 4 °C
	Precipitate can form in wells upon substrate addition when concentration of target is too high	Increase dilution factor of sample
	Using incompatible sample type (e.g. serum vs. cell extract)	Detection may be reduced or absent in untested sample types
	Sample prepared incorrectly	Ensure proper sample preparation/dilution
Large CV	Bubbles in wells	Ensure no bubbles present prior to reading plate
	All wells not washed equally/thoroughly	Check that all ports of plate washer are unobstructed/wash wells as recommended
	Incomplete reagent mixing	Ensure all reagents/master mixes are mixed thoroughly
	Inconsistent pipetting	Use calibrated pipettes & ensure accurate pipetting
	Inconsistent sample preparation or storage	Ensure consistent sample preparation and optimal sample storage conditions (e.g. minimize freeze/thaws cycles)

RESOURCES

Problem	Cause	Solution
High background	Wells are insufficiently washed	Wash wells as per protocol recommendations
	Contaminated wash buffer	Make fresh wash buffer
	Waiting too long to read plate after adding stop solution	Read plate immediately after adding stop solution
Low sensitivity	Improper storage of ELISA kit	Store all reagents as recommended. Please note all reagents may not have identical storage requirements.
	Using incompatible sample type (e.g. Serum vs. cell extract)	Detection may be reduced or absent in untested sample types

18. NOTES

UK, EU and ROW

Email: technical@abcam.com | Tel: +44-(0)1223-696000

Austria

Email: wissenschaftlicherdienst@abcam.com | Tel: 019-288-259

France

Email: supportscientifique@abcam.com | Tel: 01-46-94-62-96

Germany

Email: wissenschaftlicherdienst@abcam.com | Tel: 030-896-779-154

Spain

Email: soportecientifico@abcam.com | Tel: 911-146-554

Switzerland

Email: technical@abcam.com

Tel (Deutsch): 0435-016-424 | Tel (Français): 0615-000-530

US and Latin America

Email: us.technical@abcam.com | Tel: 888-77-ABCAM (22226)

Canada

Email: ca.technical@abcam.com | Tel: 877-749-8807

China and Asia Pacific

Email: hk.technical@abcam.com | Tel: 400 921 0189 / +86 21 2070 0500

Japan

Email: technical@abcam.co.jp | Tel: +81-(0)3-6231-0940

www.abcam.com | www.abcam.cn | www.abcam.co.jp