Human C3c ELISA Assay Kit

Catalog Number:

C3C31-K01 (1 x 96 wells)

C3C31-K02 (2 x 96 wells)

For Research Use Only. Not for use in diagnostic procedures.

v. 01.15 *(effective 05May23)*

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INTENDED USE

The Human C3c ELISA Assay Kit is to be used for the in vitro quantitative determination of human C3c in serum and plasma. This Human C3c ELISA Assay Kit is intended for laboratory research use only and is not for use in diagnostic or therapeutic procedures. The analysis should be performed by trained laboratory professionals.

INTRODUCTION

The complement system mediates a number of essential biological functions that participate in host defense against infection, initiation of the inflammatory reaction, processing and clearance of immune complexes and regulation of the immune response. There are three pathways of complement activation: the classical pathway is initiated by immune complexes, the lectin pathway by surface bound mannan binding lectin and the alternative pathway by all the surfaces that are not specifically protected against it. Each complement pathway generates a C3 convertase, a serine protease that cleaves the central complement protein C3, and generates the major cleavage fragment C3b. C3b is an opsonin and part of one of the main convertases that drives the complement cascade. In the presence of complement regulatory molecules C3b may be further degraded sequentially to iC3b, C3c, C3dg and C3d. The disadvantage of most complement biomarkers is their short half-life, making reliable sample collection and measurements difficult. Unlike other C3 fragments, C3c does not bind to other structures like pathogens, cell surface (receptors) and other plasma proteins. Therefore, C3c is a stable complement biomarker which will appear in the fluid phase only, without interference of other C3 based products. The measurement of C3c provides evidence of (uncontrolled) complement activation and can be used as an indicator of an inflammatory state. The complement is a key element of the innate immune system. Inappropriate activation is pathologic and leads for example to various autoimmune diseases (e.g. HUS). There are also indications that C3 is associated with the cardiovascular system and Parkinson's disease, making C3c a reliable and useful biomarker. The C3c ELISA, based on a antibody highly specific for a epitope exclusively on C3c, is a straightforward ELISA which can be used in diseases where complement activation is an element in an inflammatory response.

KIT FEATURES

- Working time of 3½ hours.
- Minimum concentration which can be measured is 1.6 ng/ml.
- Measurable concentration range of 1.6 – 100 ng/ml.
- Working volume of 100 µl.

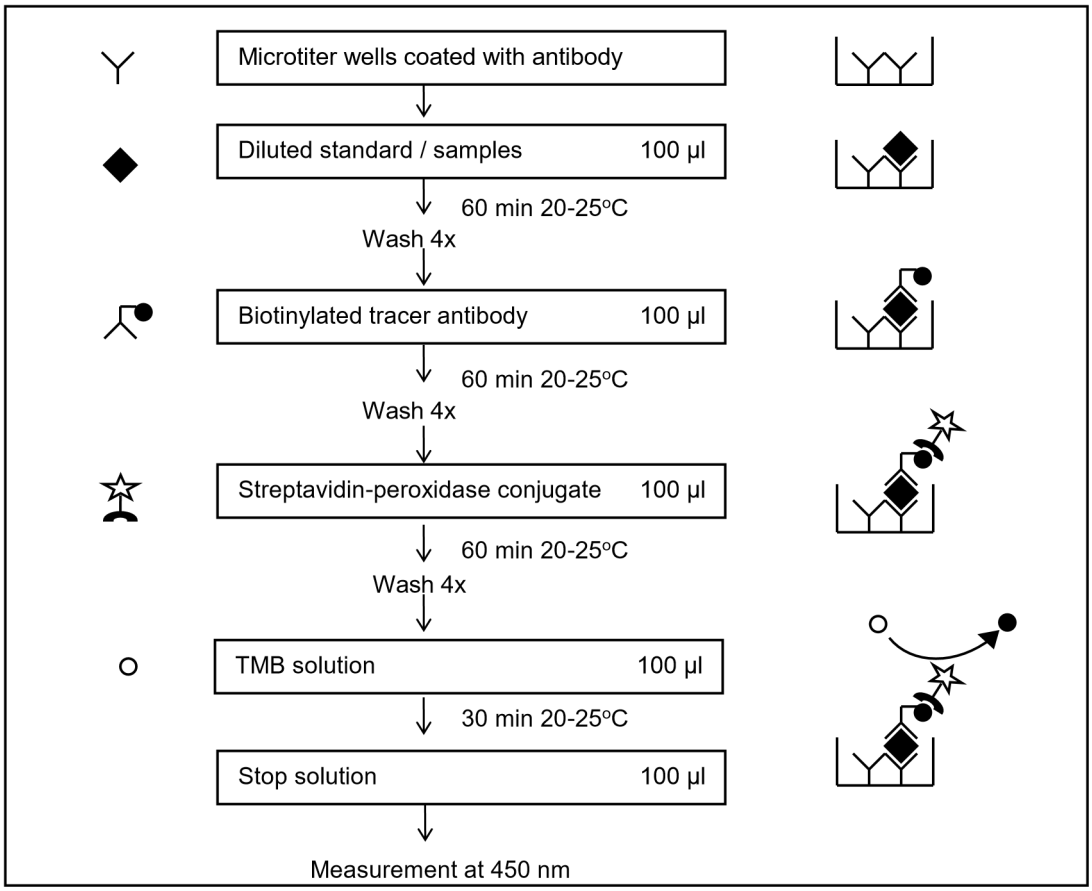
Cross-reactivity

Potential cross-reacting proteins detected in the human C3c ELISA:

Cross reactant	Reactivity
Human C3	No
Human C3a	No
Human C3b	No
Mouse C3c	No
Rat C3c	No
Pig C3c	No
Rabbit C3c	No

Table 1 Cross-reactivity for other species or proteins/peptides has not been tested.

PROTOCOL OVERVIEW



- The human C3c ELISA is a ready-to-use solid-phase enzyme-linked immunosorbent assay based on the sandwich principle with a working time of 3½ hours.
- The efficient format of a plate with twelve disposable 8-well strips allows free choice of batch size for the assay.
- Samples and standards are incubated in microtiter wells coated with antibodies recognizing human C3c.
- Biotinylated tracer antibody will bind to the captured human C3c.
- Streptavidin-peroxidase conjugate will bind to the biotinylated tracer antibody.
- Streptavidin-peroxidase conjugate will react with the substrate, tetramethylbenzidine (TMB).
- The enzyme reaction is stopped by the addition of oxalic acid.
- The absorbance at 450 nm is measured with a spectrophotometer. A standard curve is obtained by plotting the absorbance (linear) versus the corresponding concentrations of the human C3c standards (log).
- The human C3c concentration of samples, which are run concurrently with the standards, can be determined from the standard curve.

KIT COMPONENTS AND STORAGE INSTRUCTIONS

Kit component	Quantity C3C31-K01	Quantity C3C31-K02	Color code
Wash buffer 20x	1 vial (60 ml)	1 vial (60 ml)	Colorless
Dilution buffer A 20x	1 vial (7.5 ml)	1 vial (7.5 ml)	Green
Dilution buffer B 5x	1 vial (30 mL)	1 vial (30 mL)	Green
Standard	2 vials, lyophilized	4 vials, lyophilized	White
Tracer, biotinylated	1 vial, 1 ml lyophilized	2 vials, 1 ml lyophilized	White
Streptavidin-peroxidase 100x	1 tube, 0.25 ml in solution	1 tube, 0.25 ml in solution	Brown
TMB substrate	1 vial (11 ml)	1 vial (22 ml)	Brown
Stop solution	1 vial (22 ml)	1 vial (22 ml)	Red
12 Microtiter strips, pre-coated	1 plate	2 plates	
Certificate of Analysis	1	1	
Manual	1	1	
Data collection sheet	2	2	

Table 2

- Upon receipt, store individual components at 2 - 8°C. Do not freeze.
- Do not use components beyond the expiration date printed on the kit label.
- The standard and tracer in lyophilized form and the streptavidin-peroxidase in concentrated solution are stable until the expiration date indicated on the kit label, if stored at 2 - 8°C.
- The exact amount of the standard is indicated on the label of the vial and the Certificate of Analysis.
- The standard is single use. After reconstitution the standard cannot be stored.
- Once reconstituted the tracer is stable for 1 month if stored at 2 - 8°C.
- The streptavidin-peroxidase can only be stored in concentrated solution and is not stable when stored diluted.
- Upon receipt, foil pouch around the plate should be vacuum-sealed and unpunctured. Any irregularities to aforementioned conditions may influence plate performance in the assay.
- Return unused strips immediately to the foil pouch containing the desiccant pack and reseal along the entire edge of the zip-seal. Quality guaranteed for one month if stored at 2 - 8°C.

Materials required but not provided

- Calibrated micropipettes and disposable tips.
- Distilled or de-ionized water.
- Plate washer: automatic or manual.
- Polypropylene tubes.
- Calibrated ELISA plate reader capable of measuring absorbance at 450 nm.
- Adhesive covers can be ordered separately. Please contact your local distributor.
- Centrifuge for 1 ml tubes.

WARNINGS AND PRECAUTIONS

For research use only, not for diagnostic or therapeutic use.

- This kit should only be used by qualified laboratory staff.
- Do not under any circumstances add sodium azide as preservative to any of the components.
- Do not use kit components beyond the expiration date.

- Do not mix reagents from different kits and lots. The reagents have been standardized as a unit for a given lot. Use only the reagents supplied by manufacturer.
- The assay has been optimized for the indicated standard range. Do not change the standard range.
- Open vials carefully: vials are under vacuum.
- It is advised to spin down streptavidin-peroxidase tubes before use.
- Do not ingest any of the kit components.
- Kit reagents contain 2-chloroacetamide as a preservative. 2-Chloroacetamide is harmful in contact with skin and toxic if swallowed. In case of accident or if you feel unwell, seek medical advice immediately.
- The TMB substrate is light sensitive, keep away from bright light. The solution should be colorless until use.
- The stop solution contains 2% oxalic acid and can cause irritation or burns to respiratory system, skin and eyes. Direct contact with skin and eyes should be strictly avoided. If contact occurs, rinse immediately with plenty of water and seek medical advice.
- Incubation times, incubation temperature and pipetting volumes other than those specified may give erroneous results.
- Do not reuse microwells or pour reagents back into their bottles once dispensed.
- Handle all biological samples as potentially hazardous and capable of transmitting diseases.
- Hemolyzed, hyperlipemic, heat-treated or contaminated samples may give erroneous results.
- Use polypropylene tubes for preparation of standard and samples. Do not use polystyrene tubes or sample plates.
- The standard is of human origin. It was tested for various viruses and found negative. Since no test method can offer complete assurance that infectious agents are absent, this reagent should be handled as any potentially infectious human serum or blood specimen. Handle all materials in contact with this reagent according to guide-lines for prevention of transmission of blood-borne infections.

SAMPLE PREPARATION

Collection and handling

Serum

Allow freshly collected blood to clot by standing tubes vertically at room temperature for 60 minutes. Centrifuge the clotted blood (1500xg at 4oC for 15 min). Transfer the serum to a fresh polypropylene tube.

Plasma

The blood sample should be collected directly into tubes containing EDTA, citrate or heparin and cooled to 4oC immediately, the plasma should be separated and frozen at -70oC. When applicable it is advised to use EDTA plasma.

Storage

Store samples below -20°C, preferably at -70°C in polypropylene tubes. Storage at -20°C can affect recovery of human C3c. Use samples within 10 minutes after thawing. Avoid multiple freeze-thaw cycles which may cause loss of human C3cand give erroneous results.

Do not use hemolyzed, hyperlipemic, heat-treated or contaminated samples.

Dilution procedures

Serum samples

To measure human C3c accurately it is advised to dilute serum samples at least 50x with supplied dilution buffer in polypropylene tubes.

Plasma samples

To measure human C3c accurately it is advised to dilute plasma samples at least 50x with supplied dilution buffer in polypropylene tubes.

Comment regarding recommended sample dilution

The mentioned dilution for samples should be used as a guideline. The recovery of human C3c from an undiluted sample is not 100% and may vary from sample to sample. When testing less diluted samples it is advisable to run recovery experiments to determine the influence of the matrix on the detection of human C3c.

Do not use polystyrene tubes or sample plates for preparation or dilution of the samples.

Guideline for dilution of samples

Please see the table 3 for recommended sample dilutions. Volumes are based on a total volume of at least 230 µL of diluted sample, which is sufficient for one sample in duplicate in the ELISA. For dilution of samples we recommend to use at least 10 µl of sample.

Dilution		Pre-dilution	Amount of sample or pre-dilution required	Amount of Dilution buffer required
1.	10x	Not necessary	25 µl (sample)	225 µl
2.	20x	Not necessary	15 µl (sample)	285 µl
3.	50x	Not necessary	10 µl (sample)	490 µl
4.	100x	Not necessary	10 µl (sample)	990 µl
5.	500x	Recommended: 10x (see nr.1)	10 µl (pre-dilution)	490 µl
6.	1000x	Recommended: 10x (see nr.1)	10 µl (pre-dilution)	990 µl
7.	2000x	Recommended: 20x (see nr.2)	10 µl (pre-dilution)	990 µl
8.	5000x	Recommended: 50x (see nr.3)	10 µl (pre-dilution)	990 µl

Table 3

REAGENT PREPARATION

Allow all the reagents to equilibrate to room temperature (20 – 25°C) prior to use. Return to proper storage conditions immediately after use.

Wash buffer

Prepare wash buffer by mixing 60 ml of 20x wash buffer with 1140 ml of distilled or de-ionized water, which is sufficient for 2 x 96 tests. In case less volume is required, prepare the desired volume of wash buffer by diluting 1 part of the 20x wash buffer with 19 parts of distilled or deionized water.

Dilution buffer A and B

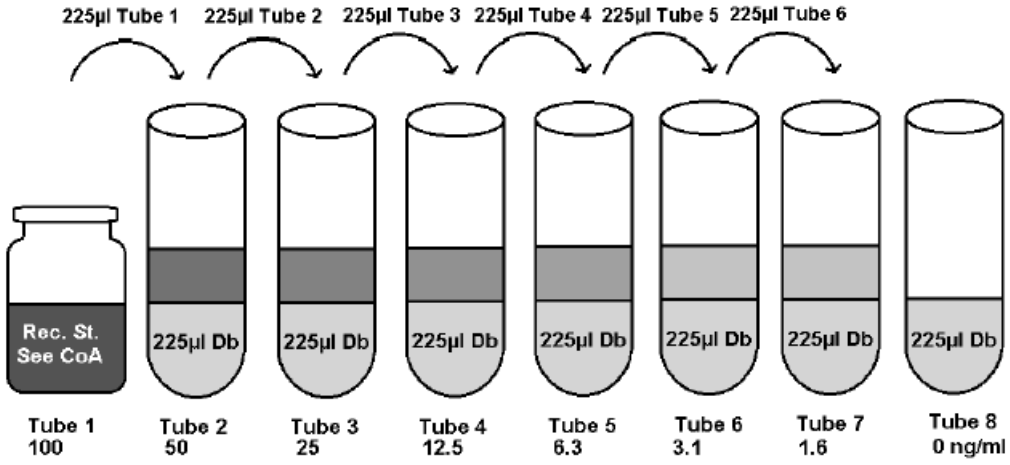
Prepare dilution buffer by mixing 7.5 mL of the 20x dilution buffer A and 30mL of 5x dilution buffer B with 112.5 mL distilled or de-ionize water, which is sufficient for 2 x 96 tests. The dilution buffer is sufficient for 2 x 96 tests.

In case less volume is required, prepare the desired volume of dilution buffer by dilution 1 part of the 20x dilution buffer A and 4 parts of dilution buffer B with 15 parts distilled or de-ionize water.

Concentrate buffer may contain crystals. In case the crystals do not disappear at room temperature within 1 hour, concentrate buffer can be warmed up to 37oC. Do not shake the solution.

Standard solution

The standard is reconstituted by pipetting the amount of dilution buffer mentioned on the CoA in the standard vial. Use the standard vial as Tube 1 in Figure 1. Prepare each human C3c standard in polypropylene tubes by serial dilution of the reconstituted standard with dilution buffer as shown in Figure 1*. After reconstitution the standard cannot be stored for repeated use.



*) CoA: Certificate of Analysis; Rec.St: Reconstituted Standard; Db: dilution buffer

Figure 1

Tracer solution

The tracer is reconstituted by pipetting 1 ml distilled or de-ionized water. Dilute the reconstituted 1 ml tracer with 11 ml dilution buffer, which is sufficient for 1 x 96 tests. Where less volume is required, prepare the desired volume of tracer by diluting 1 part of the reconstituted tracer with 11 parts of dilution buffer.

Streptavidin-peroxidase solution

It is advised to spin down streptavidin-peroxidase tubes before use. Prepare the streptavidin-peroxidase solution by mixing 0.25 ml of the 100x streptavidin-peroxidase solution with 24.75 ml dilution buffer, which is sufficient for 2 x 96 tests. In case less volume is required, prepare the desired volume of streptavidin-peroxidase solution by diluting 1 part of the 100x streptavidin-peroxidase solution with 99 parts of dilution buffer.

ELISA PROTOCOL

Bring all reagents to room temperature (20 - 25°C) before use.

1. Determine the number of test wells required, put the necessary microwell strips into the supplied frame, and fill out the data collection sheet. Return the unused strips to the storage bag with desiccant, seal and store at 2 - 8°C.
2. Transfer 100 µl in duplicate of standard, samples, or controls into appropriate wells. Do not touch the side or bottom of the wells.
3. Cover the tray and tap the tray to eliminate any air bubbles. Be careful not to splash liquid onto the cover.
4. Incubate the strips or plate for 1 hour at room temperature.
5. Wash the plates 4 times with wash buffer as follows*:
   a. Remove the cover, avoid splashing.
   b. Empty the plate by inverting plate and shaking contents out over the sink, keep inverted and tap dry on a thick layer of tissues.
   c. Add 200 µl of wash buffer to each well, wait 20 seconds, empty the plate as described in 5b.
   d. Repeat the washing procedure 5b/5c three times.
   e. Empty the plate and gently tap on thick layer of tissues.
6. Add 100 µl of diluted tracer to each well using the same pipetting order as applied in step

2. Do not touch the side or bottom of the wells.

7. Cover the tray and incubate the tray for 1 hour at room temperature.
8. Repeat the wash procedure described in step 5.
9. Add 100 µl of diluted streptavidin-peroxidase to each well, using the same pipetting order as applied in step 2. Do not touch the side or bottom of the wells.
10. Cover the tray and incubate the tray for 1 hour at room temperature.
11. Repeat the wash procedure described in step 5.
12. Add 100 µl of TMB substrate to each well, using the same pipetting order as applied in step 2. Do not touch the side or bottom of the wells.
13. Cover the tray and incubate the tray for 30 minutes at room temperature. It is advised to control the reaction on the plate regularly. In case of strong development the TMB reaction can be stopped sooner. Avoid exposing the microwell strips to direct sunlight. Covering the plate with aluminum foil is recommended.
14. Stop the reaction by adding 100 µl of stop solution with the same sequence and timing as used in step 12. Mix solutions in the wells thoroughly by gently swirling the plate. Gently tap the tray to eliminate any air bubbles trapped in the wells.
15. Read the plate within 30 minutes after addition of stop solution at 450 nm using a plate reader, following the instructions provided by the instrument's manufacturer.

*) In case plate washer is used, please note: use of a plate washer can result in higher background and decrease in sensitivity. We advise validation of the plate washer with the manual procedure. Make sure the plate washer is used as specified for the manual method.

INTERPRETATION OF RESULTS

- Calculate the mean absorbance for each set of duplicate standards, control and samples.
- If individual absorbance values differ by more than 15% from the corresponding mean value, the result is considered suspect and the sample should be retested.
- The mean absorbance of the zero standard should be less than 0.3.
- Create a standard curve using computer software capable of generating a good curve fit. The mean absorbance for each standard concentration is plotted on the vertical (Y) axis versus the corresponding concentration on the horizontal (X) axis (logarithmic scale).
- If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.
- Samples that give a mean absorbance above the absorbance for the highest standard concentration are out of range of the assay. These samples should be retested at a higher dilution.

TECHNICAL HINTS

- User should be trained and familiar with ELISA assays and test procedure.
- If you are not familiar with the ELISA technique it is recommended to perform a pilot assay prior to evaluation of your samples. Perform the assay with a standard curve only following the instructions.
- Improper or insufficient washing at any stage of the procedure will result in either false positive or false negative results. Completely empty wells before dispensing wash buffer, fill with wash buffer as indicated for each cycle and do not allow wells to sit uncovered or dry for extended periods.
- Since exact conditions may vary from assay to assay, a standard curve must be established for every run. Sample should be referred to the standard curve prepared on the same plate.
- Do not mix reagents from different batches, or other reagents and strips. Remainders should not be mixed with contents of freshly opened vials.

- Each time the kit is used, fresh dilutions of standard, sample, tracer, streptavidin-peroxidase and buffers should be made.
- Caps and vials are not interchangeable. Caps should be replaced on the corresponding vials.
- To avoid cross-contamination, change pipette tips between reagent additions of each standard, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.
- The waste disposal should be performed according to your laboratory regulations.

Technical support

Do not hesitate to contact our technical support team at info@eaglebio.com for inquiries and technical support regarding the human C3c ELISA.

QUALITY CONTROL

The Certificate of Analysis included in this kit is lot-specific and is to be used to verify results obtained by your laboratory. The absorption values provided on the Certificate of Analysis are to be used as a guideline only. The results obtained by your laboratory may differ.
This assay is designed to eliminate interference by soluble receptors, binding proteins, and other factors present in biological samples. Until all factors have been tested in the Eagle Biosciences, Inc. immunoassay, the possibility of interference cannot be excluded. For optimal performance of this kit, it is advised to work according to good laboratory practice.

TROUBLESHOOTING

Warranty claims and complaints in respect of deficiencies must be logged before expiry date of the product. A written complaint containing lot number of the product and experimental data should be sent to info@eaglebio.com.

Suggestions summarized below in Table 4 can be used as a guideline in the case of unexpected assay results.

Low absorbance	High absorbance	Poor duplicates	All wells positive	All wells negative	Possible cause
□	□		□	□	Kit materials or reagents are contaminated or expired
□					Incorrect reagents used
□		□	□		Lyophilized reagents are not properly reconstituted
□	□	□	□	□	Incorrect dilutions or pipetting errors
□		□			Improper plastics used for preparation of standard and/or samples
□	□				Improper incubation times or temperature
		□			Especially in case of 37°C incubation: plates are not incubated uniformly
□					Assay performed before reagents were brought to room temperature
□	□	□	□	□	Procedure not followed correctly
				□	Omission of a reagent or a step
		□			Poor mixing of samples
	□		□		Low purity of water

	□	□		Strips were kept dry for too long during/after washing
	□	□	□	Inefficient washing
	□	□		Cross-contamination from other samples or positive control
		□	□	TMB solution is not clear or colorless
□	□			Wrong filter in the microtiter reader
	□	□		Airbubbles
		□		Imprecise sealing of the plate after use
□				Wrong storage conditions
□				Lamp in microplate reader is not functioning optimally

Table 4

Warranty Information

Eagle Biosciences, Inc. warrants its Product(s) to operate or perform substantially in conformance with its specifications, as set forth in the accompanying package insert. This warranty is expressly limited to the refund of the price of any defective Product or the replacement of any defective Product with new Product. This warranty applies only when the Buyer gives written notice to the Eagle Biosciences within the expiration period of the Product(s) by the Buyer. In addition, Eagle Biosciences has no obligation to replace Product(s) as result of a) Buyer negligence, fault, or misuse, b) improper use, c) improper storage and handling, d) intentional damage, or e) event of force majeure, acts of God, or accident.

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For further information about this kit, its application or the procedures in this kit, please contact the Technical Service Team at Eagle Biosciences, Inc. at info@eaglebio.com or at 866-411-8023.