EAGLE BIOSCIENCES

Human C3a ELISA Kit

Catalog Number:
HCA39-K01 (1 x 96 wells)
HCA39-K02 (2 x 96 wells)
For Research Use Only. Not for use in diagnostic procedures.
v. 1.0

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INTENDED USE

The Eagle Biosciences Human C3a ELISA kit is to be used for the quantitative determination of human C3a/C3a-desArg in serum, plasma, bronchoalveolar lavage fluid (BALF) and urine samples. The Eagle Biosciences Human C3a ELISA Kit is for research use only and not for diagnostic or therapeutic procedures.

INTRODUCTION

The complement system is an important factor in innate immunity. The third complement component, C3, is central to the classical, alternative and lectin pathways of complement activation. The synthesis of C3 is tissue-specific and is modulated in response to a variety of stimulatory agents. During complement activation, C3 is proteolytically cleaved resulting in release of the anaphylatoxic peptide C3a.

C3a is a small polypeptide consisting of 74 amino acids. C3a itself is very short-lived and in serum cleaved rapidly into the more stable C3a-desArg (also called acylation stimulating protein, ASP) . Therefore, measurement of C3a-desArg allows reliable conclusions about the level of complement activation in the samples. For convenience, both forms will be referred to in the following text as C3a.

C3a is a mediator of local inflammatory processes. It induces smooth muscle contraction, increases vascular permeability, and causes histamine release from mast cells and basophilic leukocytes. C3a is involved in inflammatory reactions seen in gram-negative bacterial sepsis, trauma, ischemic heart disease, post-dialysis syndrome and a variety of autoimmune diseases. Normal values in plasma of control persons range between 48 – 150 ng/ml (median: 86.4 ng/ml, SD: 29.3 ng/ml).

Activation products of the complement cascade contain neo-epitopes that are not present in the individual native components. The human C3a ELISA kit is based on a catching monoclonal antibody that recognizes a neo-epitope on C3a-desArg. This prevents cross-reactivity with C3.

PRINCIPLE OF THE ASSAY

The human C3a 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 2 plates 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 C3a. Biotinylated tracer antibody will bind to captured human C3a. 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 C3a standards (log). The human C3a concentration of samples, which are run concurrently with the standards, can be determined from the standard curve.

KIT FEATURES

- Working time of 3 ½ hours.
- Minimum concentration which can be measured is 31.3 pg/ml.
- Measurable concentration range of 31.3 to 2000 pg/ml.
- Working volume of 100 100 µl/well.

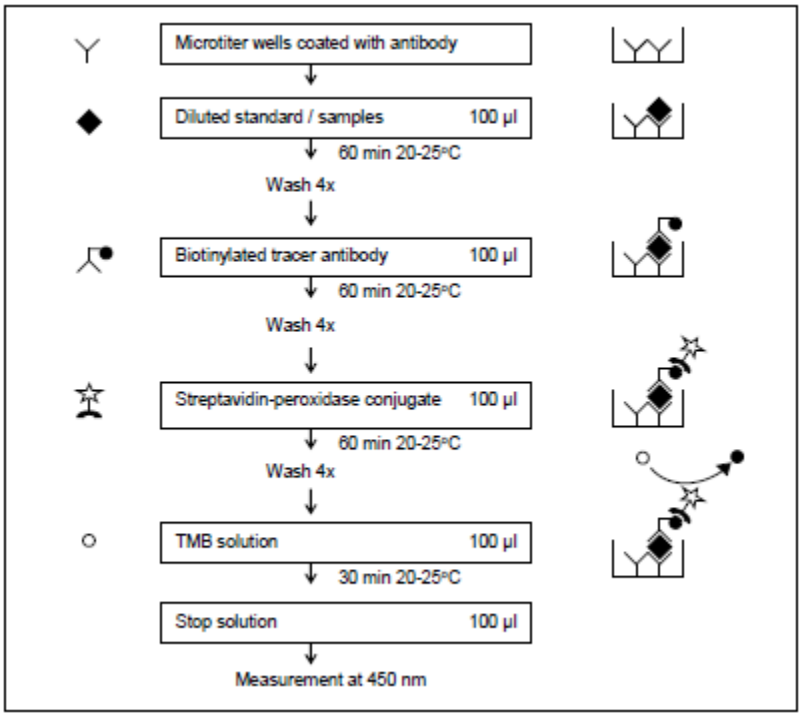
CROSS REACTIVITY

Potential cross-reacting proteins detected in the human C3a ELISA:

Cross reactant	Reactivity
Human C5a	No

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

PROTOCOL OVERVIEW



- The human C3a 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 C3a.
- Biotinylated tracer antibody will bind to captured human C3a.
- 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 standards (log).
- The human C3a 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 HCA39-K01	Quantity HCA39-K02	Color Code
Wash buffer 20x	1 vial (60 ml)	1 vial (60 ml)	Colorless
Dilution Buffer 10x	1 vial (20 ml)	1 vial (20 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	

- 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.
- After reconstitution the standard is ready to use and 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 1 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 micro wells 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 or plasma

It is critical that sample collection is performed correctly. Care must be taken to avoid C3a generation in the samples. All specimen handling operations should be carried out at 4°C for plasma and serum (immediately after clotting).

Collect blood using normal aseptic techniques. Blood samples should be kept on ice. If serum is used, separate serum from blood after clotting at room temperature within 1 hour by centrifugation (1500xg at 4°C for 15 min). Transfer the serum to a fresh polypropylene tube. If plasma is used, separate plasma from blood within 20 minutes after blood sampling by centrifugation (1500xg at 4°C for 15 min). Transfer the plasma to a fresh polypropylene tube. Assay immediately or store samples on ice for up to 6 hours before assaying. Aliquots of serum or plasma may also be stored at –70° C for extended periods of time. Avoid repeated freeze-thaw cycles

Most reliable results are obtained if EDTA plasma is used.

Bronchoalveolar lavage fluid (BALF)
Perform BALF during bronchoscopy by standardized washing of the segment. Aspirate each aliquot of physiologic fluid immediately after inspiration. Discard the first aliquot of recovered BALF. Collect the BALF in polypropylene tubes and keep it on ice. Separate cells from BALF by centrifugation (500xg at 4°C for 5 min). Filter cell free BALF through a layer of gauze to remove mucus strands.

Urine
Collect urine using normal aseptic techniques. Centrifugate the urine to remove debris (1500xg at 4 °C for 15 min). Transfer urine to a fresh polypropylene tube.

Storage
Store samples at -70°C in polypropylene tubes. Storage at -20°C can affect recovery of human C3a. Use samples within 6 hours after thawing. Avoid multiple freeze-thaw cycles which may influence the reliability of human C3a measurement and give erroneous results.
Do not use hemolyzed, hyperlipemic, heat-treated or contaminated samples.
Before performing the assay, samples should be brought to room temperature (18 – 25°C) and mixed gently. Prepare all samples (controls and test samples) prior to starting the assay procedure. Avoid foaming.

Dilution procedures

Serum samples
Human C3a can be measured accurately if serum samples are diluted at least 4000x with supplied dilution buffer in polypropylene tubes.

Plasma samples
Human C3a can be measured accurately if plasma samples are diluted at least 300x with supplied dilution buffer in polypropylene tubes.

BALF samples
Human C3a can be measured accurately if BALF samples are diluted at least 5x with supplied dilution buffer in polypropylene tubes.

Urine samples
Human C3a can be measured accurately if urine samples are diluted at least 4x with supplied dilution buffer in polypropylene tubes.

Remark regarding recommended sample dilution
The mentioned dilution for samples is a minimum dilution and should be used as a guideline. The recovery of human C3a 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 C3a.
Do not use polystyrene tubes or sample plates for preparation or dilution of the samples.

Guideline for dilution of samples
Please see the table below 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.

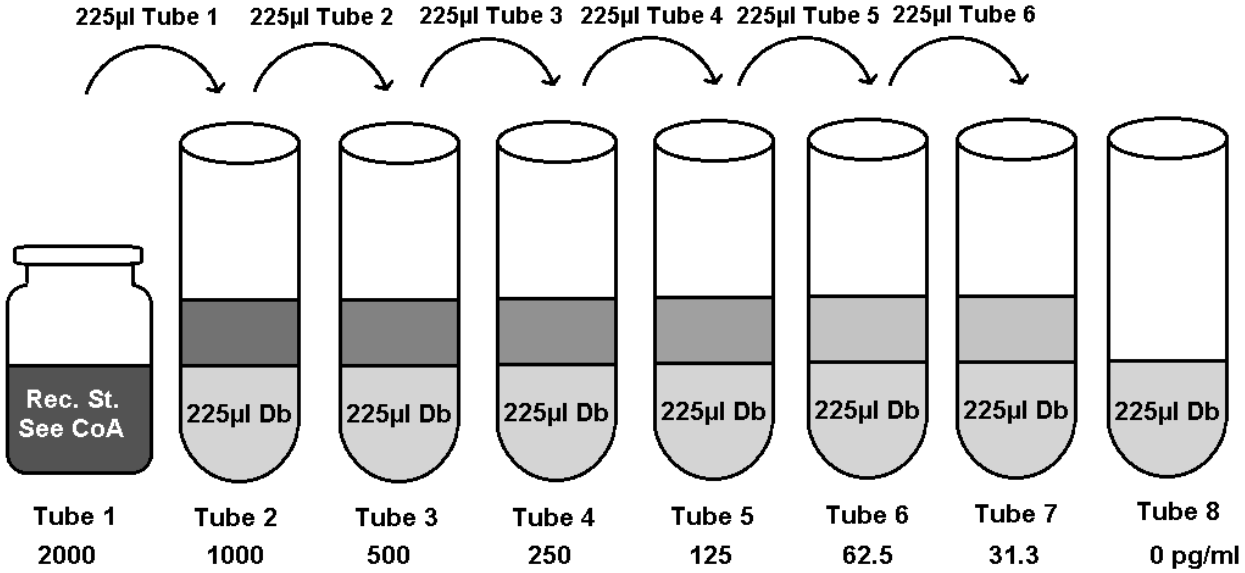
Dilution	Pre-Dilution	Amount of sample or pre-dilution required	Amount of dilution buffer required
10x	Not necessary	25 µl (sample)	225 µl
20x	Not necessary	15 µl (sample)	285 µl
50x	Not necessary	10 µl (sample)	490 µl
100x	Not necessary	10 µl (sample)	990 µl
500x	Recommended: 10x (see nr. 1)	10 µl (pre-dilution)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

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 de-ionized water.

Dilution buffer
Prepare dilution buffer by mixing 20 ml of the 10x dilution buffer with 180 ml of distilled or de-ionized water, which is sufficient for 2x 96 tests. In case less volume is required, prepare the desired volume of dilution buffer by diluting 1 part of the 10x dilution buffer with 9 parts of distilled or de-ionized water. Concentrated dilution buffer may contain crystals. In case the crystals do not disappear at room temperature within 1 hour, concentrated dilution buffer can be warmed up to 37°C. 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 C3a 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.



Tracer solution
The tracer is reconstituted by pipetting 1 ml distilled or de-ionized water. Dilute the reconstituted 1 ml tracer with 11 ml wash/dilution buffer, which is sufficient for 1 x 96 tests. In case less volume is required, prepare the desired volume of tracer by diluting 1 part of the reconstituted tracer with 11 parts of wash/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 wash/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 wash/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. Carefully 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 1x 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 aluminium 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. Samples 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-contaminations, 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.

REFERENCES

1. Morad H et al; Time-course analysis of C3a and C5a quantifies the coupling between the upper and terminal Complement pathways in vitro. J Immunol Meth 2015, 427: 13
2. Hartmann H et al; Rapid quantification of C3a and C5a using a combination of chromatographic and immunoassay procedures. J Immunol Meth 1993, 166: 35
3. Stove S at al; Re-evaluation of the storage conditions for blood samples which are used for determination of complement activation. J Immunol Meth 1995, 182: 1
4. Roy, R et al; Complement component 3C3 and C3a Receptor are required in chitin-dependent allergic sensitization to Aspergillus fumigatus but dispensable in chitin-induced innate allergic inflammation. Molecular Biology 2013, 4:2
5. Sethu, S et al; Immunoglobulin G1 and immunoglobulin G4 antibodies in multiple sclerosis patients treated with IFNβ interact with the endogenous cytokine and activate complement. Clin Immunol 2013, 148: 2

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