

MicroVue™ Complement C3a Plus EIA

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

SUMMARY

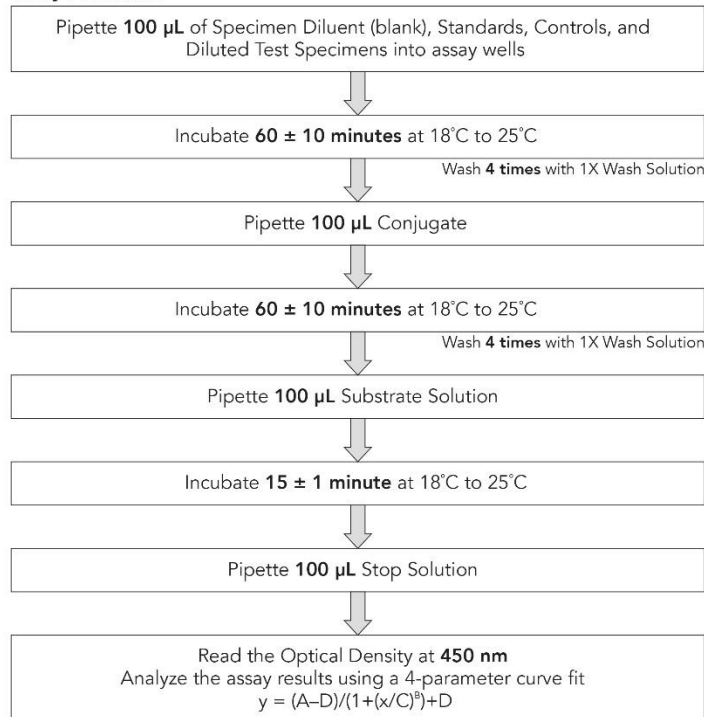
Reagents Preparation

- Dilute Wash Solution Concentrate 1:20 with DI Water.

Specimen Preparation (Plasma – 1:200; Serum – 1:5000)

- Pipette Specimen Diluent for Dilution 1 into separate tubes or plates:
90 μ L for each plasma specimen (for plasma Dilution 1)
490 μ L for each serum specimen (for serum Dilution 1)
- Pipette Specimen Diluent for Dilution 2 into separate tubes or plates:
475 μ L for each plasma specimen (for plasma Dilution 2)
495 μ L for each serum specimen (for serum Dilution 2)
- Rapidly thaw specimens by incubating at 37°C until approximately 90% of the specimen is thawed. Place immediately on ice.
- Gently mix each specimen.
- Transfer 10 μ L of each plasma or serum specimen to the corresponding plasma or serum Dilution 1, and mix gently.
- Transfer the following amounts of each Dilution 1 mixture to the specified Dilution 2, and mix gently.
25 μ L plasma Dilution 1 \rightarrow 475 μ L plasma Dilution 2
5 μ L serum Dilution 1 \rightarrow 495 μ L serum Dilution 2

Assay Procedure



INTENDED USE

The MicroVue C3a Plus Enzyme Immunoassay is for measurement of C3a.

SUMMARY AND EXPLANATION

The MicroVue C3a Plus Enzyme Immunoassay is a 96 well, direct capture immunoassay for the measurement of C3a.

Under normal conditions, activation of the classical, alternative, or lectin complement pathways results in the formation of a C3 convertase multi-molecular enzyme capable of cleaving C3 to C3a and C3b.¹ C3a is a low molecular weight (approximately 9kD) protein fragment of 77 amino acids.² C3a is rapidly metabolized by the serum enzyme carboxypeptidase N to a more stable, 76 amino acid form, C3a des-Arg.³ For convenience, both forms will be referred to as "C3a" for purposes of this documentation.

The MicroVue C3a Plus assay, a rapid, highly specific and quantitative procedure for measuring C3a levels, is designed for investigations into the role or status of complement pathway activation in numerous research settings, and for monitoring the generation of C3a *in vivo* or *in vitro*. C3a has been shown to increase vascular permeability, to be spasmogenic and chemotactic, and to induce the release of pharmacologically active mediators from a number of cell types. The role of C3a in the pathogenesis of inflammatory reactions seen in gram-negative bacterial sepsis, trauma, ischemic heart disease, cerebral ischemia, post dialysis syndrome and several autoimmune diseases (including rheumatoid arthritis, lupus erythematosus, and acute glomerulonephritis) is well documented.^{4, 6-23}

PRINCIPLE OF THE PROCEDURE

The MicroVue C3a Plus Enzyme Immunoassay is a three-step procedure utilizing (1) a microassay plate coated with a murine monoclonal antibody specific for a neo-epitope on human C3a, (2) an HRP-conjugated polyclonal antibody to the C3a region of C3, and (3) a chromogenic substrate.

In Step 1, Standards, Controls and diluted test specimens are added to the assay wells coated with a murine monoclonal antibody to C3a. The monoclonal antibody binds to C3a in the Standards, Controls or specimens. After the incubation period, a wash cycle removes any unbound material.

In Step 2, horseradish peroxidase (HRP)-conjugated anti-C3 (C3a) is added to each assay well. The enzyme conjugated anti-C3 (C3a) binds to the immobilized C3a captured in the first step. After the incubation period, a wash cycle removes any unbound conjugate.

In Step 3, 3,3',5,5' tetramethylbenzidine (TMB), a ready-to-use, chromogenic substrate solution, is added to the assay wells. The bound HRP reacts with the substrate, forming a blue color. After the incubation period, the reaction is stopped chemically, which results in a color change from blue to yellow, confirming that the reaction has taken place. The color intensity is measured spectrophotometrically at A₄₅₀. The color intensity of the reaction mixture is proportional to the concentration of C3a present in the Standards, Controls and diluted test specimens. Results are calculated from the generated standard curve using 4-parameter analysis.

REAGENTS AND MATERIALS PROVIDED

96 Assays for C3a complex

MicroVue C3a Plus EIA kit contains the following:

A C3a Standards:	Parts 5140 – 5145	1.5 mL, 1 each
B	Ready to use. Contains purified native human C3a protein with an assigned protein concentration (ng/mL),	
C	protein stabilizers	
D		
E		
L Low Control	Part 5146	1.5 mL
	Ready to use. Contains purified native human C3a protein with an assigned protein concentration (ng/mL),	
	protein stabilizers	
H High Control	Part 5147	1.5 mL
	Ready to use. Contains purified native human C3a protein with an assigned protein concentration (ng/mL),	
	protein stabilizers	
① Coated Strips	Part 5148	12 each
	Eight-well strips coated with a murine monoclonal antibody in a resealable foil pouch	
② Stop Solution	Part A9947	12 mL
	Contains 1N (4%) Hydrochloric Acid	
③ 20X Wash Solution Concentrate	Part A9957	50 mL, 2 each
	Contains phosphate buffered saline (PBS), 1.0% Tween-20® and 0.035% ProClin® 300	
④ Specimen Diluent	Part 5150	50 mL
	Contains a buffered protein base with 0.05% ProClin 300	
⑤ TMB Substrate	Part 5059	12 mL
	Ready to use. Contains 3,3',5,5'-tetramethylbenzidine (TMB) and Hydrogen Peroxide (H ₂ O ₂)	
⑥ Conjugate	Part 5151	12 mL
	Contains Horseradish Peroxidase-conjugated polyclonal antibody to C3a	

MATERIALS REQUIRED BUT NOT PROVIDED

- Timer (60-minute range)
- 96 well dilution plate (VWR REF: 47743-828) or test tubes and racks for sample dilution (optional)
- Clean, unused microassay plate for replica plate method (optional)
- Graduated container for wash buffer dilution
- Wash bottle
- Micropipettes and sterile, disposable pipette tips
- Adjustable multichannel pipette (8 or 12 channels) or repeating micropipettes
- Reagent reservoirs for adding conjugate, substrate and stop solutions to plate (use clean, unused reservoirs for each reagent)
- Plate reader capable of A₄₅₀ optical density readings between 0.0 and 3.0
- Deionized or distilled water

WARNINGS AND PRECAUTIONS

- For Research Use Only. Not for use in diagnostic procedures.
- Treat specimen samples as potentially biohazardous material. Follow Universal Precautions when handling contents of this kit and any patient samples.

- Use the supplied reagents as an integral unit prior to the expiration date indicated on the package label.
- Store assay reagents as indicated.
- Do not use Coated Strips if pouch is punctured.
- ProClin 300 is used as a preservative. Incidental contact with or ingestion of buffers or reagents containing ProClin can cause irritation to the skin, eyes or mouth. Use good laboratory practices to reduce exposure. Seek medical attention if symptoms are experienced.
- The Stop Solution is considered corrosive and can cause irritation. Do not ingest. Avoid contact with eyes, skin, and clothing. If contact is made, immediately rinse affected area with water. If ingested, call a physician.
- Each donor unit used in the preparation of the standards and control sera of this product was tested by an FDA-approved method for the presence of antibody to human immunodeficiency virus (HIV1 and HIV2) and to hepatitis C virus, as well as for hepatitis B surface antigen. Since no test method can offer complete assurance that infectious agents are absent, these reagents should be handled at Biosafety Level 2 as recommended for any potentially infectious human serum or blood specimen in the Centers for Disease Control/National Institutes of Health manual "Biosafety in Microbiological and Biomedical Laboratories."²⁴
- Use of multichannel pipettes or repeat pipettors is recommended to ensure timely delivery of reagents.
- For accurate measurement of samples, add samples and standards precisely. Pipette carefully using only calibrated equipment.
- Proper collection and storage of test specimens are essential for accurate results (see *SPECIMEN HANDLING AND PREPARATION*).
- Avoid microbial or cross-contamination of specimens or reagents.
- Test each sample in duplicate.
- Do not use any single microassay well for more than one test.
- Using incubation times and temperatures other than those indicated in the Procedure section may give erroneous results.
- The TMB Substrate must be protected from light during storage and incubation. Avoid contact with eyes, skin, and clothing. If contact is made, immediately rinse affected area with water.
- Do not allow microassay wells to dry once the assay has begun.
- When removing liquid from the microassay wells, do not scrape or touch the bottom of the wells.
- Heat-inactivated, hyperlipemic, or contaminated specimens may give erroneous results.
- To avoid aerosol formation during washing, use an apparatus to aspirate the wash fluid into a bottle containing household bleach.
- A wash bottle should be used to wash the plate (*ASSAY PROCEDURE*, Step 10). For best results, do not use a multichannel pipette to wash the microassay plate.
- Testing should be performed in an area with adequate ventilation.
- Dispose of containers and unused contents in accordance with Federal, State and Local regulatory requirements.
- Wear suitable protective clothing, gloves, and eye/face protection when handling the contents of this kit.
- Wash hands thoroughly after handling.
- For additional information on hazard symbols, safety, handling and disposal of the components within this kit, please refer to the Safety Data Sheet (SDS) located at quidel.com.

STORAGE

Store unopened kit at 2°C to 8°C. Bring reagents and materials selected for use to 18°C to 25°C before use. Place all unused microassay strips into the storage bag, reseal the bag, and store at 2°C to 8°C.

INDICATIONS OF INSTABILITY OR DETERIORATION OF REAGENTS

Cloudiness or discoloration of the diluted Wash Solution indicates a deterioration of this reagent. If this occurs, the solution should be discarded.

Cloudiness of the Specimen Diluent indicates a deterioration of this reagent. If this occurs, the solution should be discarded. The color of the specimen diluent may vary from pink to brown, which is normal and does not indicate deterioration or instability.

SPECIMEN HANDLING AND PREPARATION

Handle and dispose of all specimens using Universal Precautions.

All specimen-handling operations should be carried out at 2°C to 8°C.

Specimen Collection

The proper collection, processing and storage of specimens is essential since C3a may be generated in improperly handled specimens through artifactual complement activation. For optimal plasma results, K2 EDTA collection tubes are recommended (Fisher REF: 22 040-161).

Values for serum samples will typically be higher than those obtained with EDTA plasma samples. The C3a levels in EDTA plasma may therefore more accurately represent the *in vivo* concentrations.²⁵

Serum and EDTA plasma specimens should be collected aseptically using standard techniques.²⁶ The specimens should be tested immediately or stored on ice for no longer than two hours before being assayed.

If the specimen cannot be tested within two hours under the guidelines detailed above, the specimen should be frozen at –70°C, or below.

Specimen Stabilizing Solution (Part A9576) can also be used to prepare human serum and plasma specimens for storage. Proper use of this product, available only from Quidel, requires that the specimen be mixed 1:1 with the solution prior to freezing. Additional technical information about the solution is available upon request.

Thawing Frozen Specimens

To minimize specimen handling time, set up a dilution plate (or tubes) and add the appropriate volume of diluent (as described in the *Specimen Dilution* section below) prior to thawing specimens for evaluation.

Thaw frozen specimens rapidly at 37°C until just thawed. Transfer thawed specimens immediately to ice to prevent complement activation prior to dilution. **Keep specimens on ice for no longer than two hours. Do not leave specimens at 37°C**, as complement activation may occur. Do not thaw specimens at room temperature or on ice as this can lead to C3 activation and affect results. Specimens should be tested as soon as possible after thawing. Only one freeze/thaw cycle may be performed without affecting the samples. If samples need additional freezing for further analysis, Quidel suggests freezing multiple aliquots of the specimen to prevent multiple freeze/thaw cycles.

Specimen Dilution

CAUTION: Treat all specimens as potentially infectious. Use Universal Precautions. Do not use heat-inactivated, contaminated, or improperly stored specimens.

NOTE: See *Thawing Frozen Specimens* for important notes on proper methods to thaw frozen specimens. Proper sample handling is essential for accurate results.

CRITICAL NOTE: Perform specimen collection and dilution correctly to avoid complement activation and resultant C3a generation in specimens.

Specimens **must** be diluted so that A_{450} values observed are above the LLOQ and do not exceed the A_{450} value of the assay ULOQ. Specimens with A_{450} readings outside this range should be re-assayed at a new dilution.

Determine the number (N) of specimens to be tested. Label 2 sets of test tubes #1 through #N, and record which specimen corresponds to each tube. Alternatively, a 96-well dilution plate may be used for making the dilutions.

Prepare an appropriate dilution (see the following section) of each specimen using the Specimen Diluent. For each dilution, mix gently to avoid formation of foam and bubbles. Do not store or reuse diluted specimens.

Dilution Method

Dilute plasma specimens 1:200 in the provided Specimen Diluent.

For optimal results, perform two dilutions to prepare each sample as follows:

- For each test specimen, pipette 90 μL of Specimen Diluent for dilution 1 and 475 μL for dilution 2 into separate dilution tubes or plates.
- Prepare dilution 1 by adding 10 μL of the Test Specimen to 90 μL of Specimen Diluent (the plasma dilution 1 tube or plate). Mix gently.
- Prepare dilution 2 by adding 25 μL of dilution 1 to 475 μL of Specimen Diluent (the plasma dilution 2 tube or plate). Mix gently.

Dilute serum specimens 1:5000 in the provided Specimen Diluent as follows:

- For each test specimen, pipette 490 μL of Specimen Diluent for dilution 1 and 495 μL for dilution 2 into separate dilution tubes or plates.
- Prepare dilution 1 by adding 10 μL of the Test Specimen to 490 μL of Specimen Diluent (the serum dilution 1 tube or plate). Mix gently.
- Prepare dilution 2 by adding 5 μL of dilution 1 to 495 μL of Specimen Diluent (the serum dilution 2 tube or plate). Mix gently.

Adding Diluted Specimens to the Microtiter Wells

Complete the addition of diluted specimens to the microtiter wells within 15 minutes of the application of the first specimen. Either of two methods can be used to add diluted specimens, Standards, Controls, and buffer, to the wells (see Step 6 of *ASSAY PROCEDURE*). For small assay runs where only a few specimens are being tested, the diluted specimens and other reagents may be added directly to their assigned wells with a micropipette (100 μL /well). For small or large runs, but especially larger runs, we recommend the use of a multichannel pipettor for adding specimens as follows.

To load the Standards, Controls and diluted specimens into the microassay wells as rapidly as possible, a “replica plating” procedure can be employed. Instead of adding 100 μL of each Standard, Control, or diluted specimen to the antibody-coated wells individually, add 120–130 μL of each solution to individual wells in a blank plate (not provided) corresponding to the final EIA pattern desired. After all the solutions to be tested have been added to the microassay wells in the blank plate, rapidly transfer 100 μL from each blank well to the antibody-coated wells using a multichannel micropipettor. To avoid the possibility of cross-contamination, pipette tips must be changed each time there is a change in the composition of the samples to be transferred.

The “replica plating” procedure may be used to conveniently add the Conjugate, Substrate, and Stop Solution, as well.

REAGENT preparation

Bring all reagents and materials to 18°C to 25°C before use.

After removing the needed reagents and materials, return the unused items to their appropriate storage temperatures (*see STORAGE*).

Standards and Controls

Standards and Controls are supplied ready to use and do not require dilution or preparation prior to use.

Wash Solution

Mix the 20X Wash Solution Concentrate by inverting the bottle several times. If the 20X Wash Solution Concentrate has been stored at 2°C to 8°C, crystals may have formed. To dissolve the crystals, warm the bottle in a 37°C to 50°C water bath until all crystals have dissolved, and follow by mixing thoroughly. Prepare the Wash Solution by diluting the entire contents of one of the bottles of 20X Wash Solution Concentrate up to one liter with distilled or deionized water. Mix thoroughly. The Wash Solution is stable for 30 days when stored in a clean container at 2°C to 8°C. If discoloration or cloudiness occurs, discard the reagent.

Microassay Strips

Determine the number of wells required for the assay. Quidel recommends testing the blank wells, Controls, and Standards in duplicate. Remove the unneeded strips and place them in the storage bag, reseal the bag, and return it to storage at 2°C to 8°C. Secure the strips to be used in the assay in the assay plate frame.

ASSAY PROCEDURE

Read entire Product Insert before beginning the assay.

See REAGENT PREPARATION and WARNINGS AND PRECAUTIONS before proceeding.

1. Record the microassay well positions corresponding to the blank well(s), all test specimens, Standards, and Controls as well as the indicated lot numbers from the vial labels. Label one corner of the Microassay Plate for orientation.
2. Label dilution plate/tubes to correspond to all test specimens.
3. Add Specimen Diluent to the dilution plate/tubes.
4. Thaw test specimens and dilute immediately.
5. Select one or more wells to serve as a blank. Add 100 µL of Specimen Diluent to the well(s) that will be used to blank the plate reader.
6. Add 100 µL of each C3a Standard (A, B, C, D, E) to duplicate wells. **NOTE: The Standards are ready to use and do not need dilution.**
7. Add 100 µL of both the C3a Low Control and C3a High Control to duplicate wells. **NOTE: The Controls are ready to use and do not need dilution.**
8. Add 100 µL of each diluted specimen to its assigned microassay well. (*See Specimen Dilution*).
9. Incubate at 18°C to 25°C for 60 ± 10 minutes.
10. Wash the microassay wells as follows:
 - a. After the incubation in step 9 remove the liquid from each well.
 - b. Add approximately 300 µL Wash Solution to each well using a wash bottle or automated filling device.
 - c. Remove the liquid from each well, and tap firmly on absorbent paper (if manual washing is used).
 - d. Repeat steps b-c three additional times for a total of four washes.**
 - e. After the fourth wash cycle, invert the plate, and tap firmly on absorbent paper twice to remove any remaining liquid.
11. Using a multichannel or repeating pipette, dispense 100 µL of C3a Conjugate into each washed test well, including the blank well(s).
12. Incubate the microassay strips at 18°C to 25°C for 60 ± 10 minutes.

13. Wash the microassay wells after the 60-minute incubation (step 12), as described under *ASSAY PROCEDURE*, step 10.
14. Immediately following the wash procedure, dispense 100 μ L of the Substrate Solution into each well, including the blank(s).
15. Incubate the microassay strips at 18°C to 25°C for 15 (\pm 1) minutes.
16. Add 100 μ L of Stop Solution to each well to stop the enzymatic reaction. The Stop Solution should be added to the wells in the same order and at the same rate as the Substrate Solution. Gently tap the plate to disperse the color development evenly. **NOTE: Optimal results may be obtained by using the plate reader's auto-mix function (if available) just prior to reading the plate.**
17. Determine the absorbance reading at 450 nm (A_{450} value) for each test well within 60 minutes after the addition of the Stop Solution (step 16), making the necessary blank correction.
18. Determine the concentration of samples and Controls from the standard curve.
19. Dispose of the remaining diluted specimens and controls and the used microassay strips (see *WARNINGS AND PRECAUTIONS*).

QUALITY CONTROL

Good laboratory practice recommends use of controls to ensure that the assay is performing properly. Each C3a Plus kit contains Low and High Controls that can be used for this purpose. Control ranges are provided. The Control values are intended to verify the validity of the curve and sample results. Each laboratory should establish its own parameters for acceptable assay limits. If the control values are NOT within your laboratory's acceptance limits, the assay results should be considered questionable, and the samples should be repeated. In addition, the package insert requires that the standard curve generated with the kit Standards meet stringent validation requirements. If the assay does not meet these requirements, repeat the assay, or contact Quidel Technical Service.

The Certificate of Analysis included in this kit is lot specific and is to be used to verify that the results obtained by your laboratory are similar to those obtained at Quidel Corporation. The optical density values provided are to be used as a guideline only. The results obtained by your laboratory may differ.

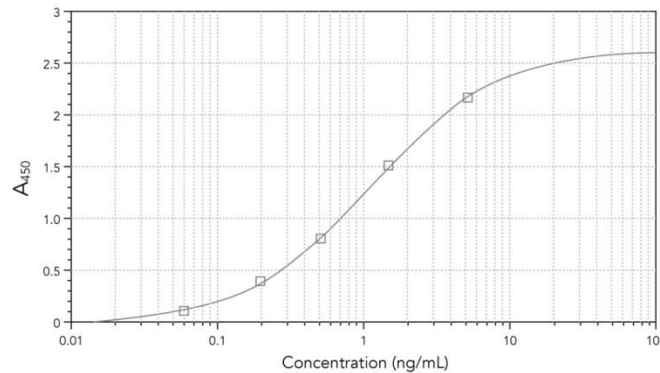
INTERPRETATION OF RESULTS

Calculation of Results

Use of the Standard Curve: The standard curve for the C3a Plus EIA is generated using the blank subtracted A_{450} values for each Standard (on the y axis) and the assigned concentration for each Standard (on the x axis). The standard curve must meet the Validation Requirements. Most computers and calculators are capable of performing these calculations. An example of a typical standard curve is shown in Figure 1.

Representative Standard Curve

Figure 1



Calculation of Actual C3a Concentration in Specimens

The assigned concentration on the certificate of analysis are absolute units of C3a. The concentration of C3a in a specimen is determined by multiplying the determined concentration by the appropriate specimen dilution factor. For example, if an EDTA-plasma specimen is diluted 1:200 for the assay, and the 4-Parameter logistic curve yields a concentration of 0.5 ng C3a/mL, then the concentration of C3a in the specimen would be 100 ng C3a/mL (or 200 x 0.5).

When test specimens yield A_{450} values greater than the ULOQ or less than the LLOQ, obtain a more accurate C3a concentration by re-assaying the test specimens at a different dilution so the new A_{450} values will be within these limits. All repeat assays must include a retest of the C3a Standards and Controls.

Validation

Determine the upper asymptote (D) and correlation coefficient of the derived 4-parameter logistic curve fit for the C3a Plus A, B, C, D and E Standards. The values must fall within the specified ranges to qualify the assay:

correlation coefficient (r): > 0.98

Upper asymptote (D): ≥ 1.49

Refer to the certificate of analysis for the acceptable C3a concentration range for the Low and High Controls.

LIMITATIONS OF THE PROCEDURE

The MicroVue C3a Plus Enzyme Immunoassay has been used to test specimens collected as serum or as plasma in K2 EDTA. Other anticoagulants have not been tested.

PERFORMANCE CHARACTERISTICS

Limits

LOD: The limit of detection (LOD) for the C3a Plus assay is 0.012 ng/mL, determined by the upper 3SD limit in a zero standard study.

LLOQ: The lower limit of quantitation (LLOQ) for the C3a Plus assay is 0.023 ng/mL, the lowest concentration on the standard curve that met internal criteria for accuracy and precision.

ULOQ: The upper limit of quantitation (ULOQ) for the C3a Plus assay is 2.531 ng/mL, the highest concentration on the standard curve that met internal criteria for accuracy and precision. Diluted samples with a concentration above this limit should be retested at a higher dilution.

Interfering Substances

The following substances were tested in the C3a Plus assay and found to not interfere with the assay:

Substance	Concentration
Albumin	6000 mg/dL
γ Globulin	6000 mg/dL
Bilirubin	20 mg/dL
Hemoglobin	200 mg/dL
Triglycerides	3000 mg/dL
Na + Heparin	3 U/dL
Glucose	1000 mg/dL
Cholesterol	500 mg/dL
EDTA	10 mM
Whole C3	5 µg/mL
Whole C5	5 µg/mL
C5a	5 µg/mL

Precision

Intra- and Inter-assay precision was determined by assaying 20 replicates of 2 plasma samples and 2 serum samples in 10 different assays.

Sample	C3a (ng/mL)	Intra-assay ¹ C.V. (%)	Intra-assay ² C.V. (%)
EDTA Plasma	55.80	4.7	14.7
	119.5	5.0	19.6
Serum	533.3	5.3	8.3
	2308	4.5	5.9

¹n = 20 replicates ²n = 10 assays

Linearity

Linearity was performed by diluting samples with specimen diluent and comparing observed values with expected values.

Sample	Dilution Factor	Observed C3a (ng/mL)	Recovery (%)
EDTA Plasma	175	67.88	105.5
	200	64.36	100.0
	225	63.67	98.9
	250	62.99	97.9
	275	65.13	101.2
	300	65.54	101.8
Serum	1,250	2097	96.5
	2,500	2128	97.9
	5,000	2173	100.0
	10,000	2160	99.4
	20,000	2196	101.1
	40,000	2423	111.5

Spike Recovery

Spike Recovery was performed by spiking samples with a known quantity of purified C3a and comparing observed values with expected values.

Sample	C3a (ng/mL)	Spike (ng/mL)	Result (ng/mL)	Recovery (%)
Serum 1	1021		2788	99.7
Serum 2	615.1	1775	2343	98.0
Serum 3	2080		3677	95.4
Plasma 1	53.4		230.5	99.7
Plasma 2	87.4	177.8	240.7	90.8
Plasma 3	118.3		278.8	94.1

ASSISTANCE

To place an order or for technical support, please contact a Quidel Representative at 800.874.1517 (in the U.S.) or 858.552.1100 (outside the U.S.), Monday through Friday, from 8:00 a.m. to 5:00 p.m., Eastern Time. Orders may also be placed by fax at (740) 592-9820. For e-mail support contact customerservice@quidel.com or technicalsupport@quidel.com.

For services outside the U.S.A., please contact your local distributor. Additional information about Quidel, our products, and our distributors can be found on our website quidel.com.

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REF

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GLOSSARY

REF

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LOT

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Use by



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Temperature limitation



Consult e-labeling
instructions for use

RUO

For Research use only



Contains sufficient for 96 determinations

CONT

Contents/Contains

CONTROL

Control
