



# MicroVue™ Complement

C4a Fragment EIA

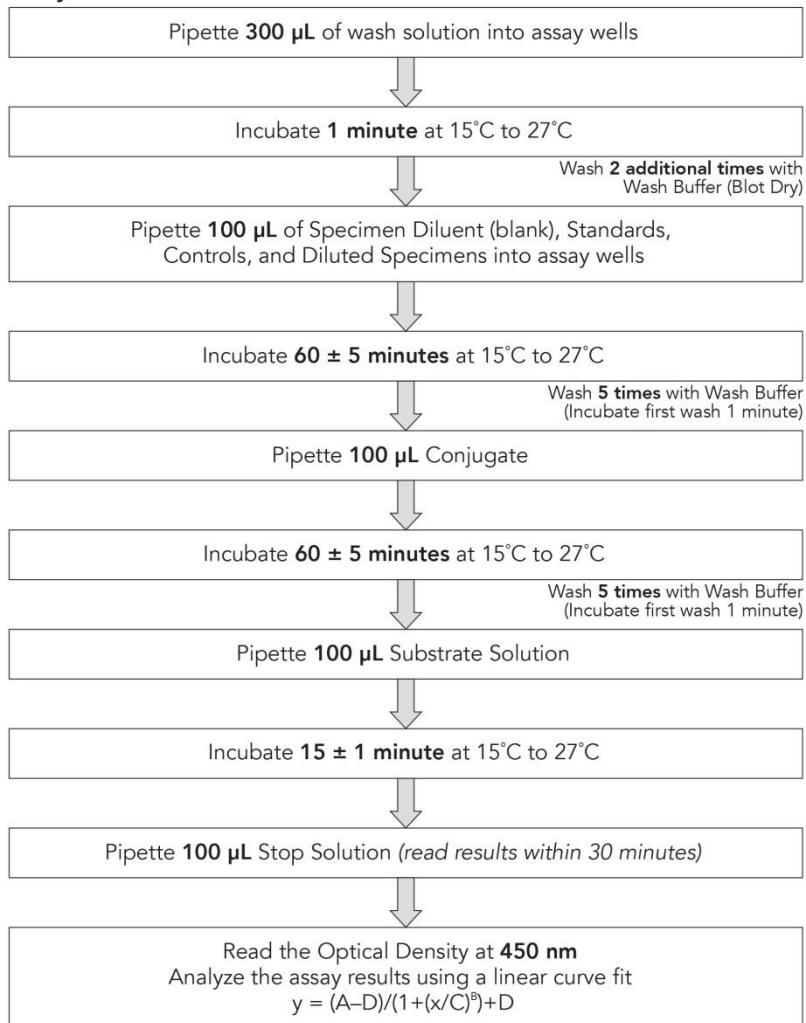
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## SUMMARY

### Reagent and Sample Preparation

- Dilute Wash Buffer Concentrate 1:20 with DI Water.
- Dilute Plasma Samples 1:40 with Specimen Diluent (e.g. 10  $\mu$ L sample + 390  $\mu$ L diluent).
- Dilute Serum Samples 1:80 with Specimen Diluent (e.g. 10  $\mu$ L sample + 790  $\mu$ L diluent).

### Assay Procedure



## SUMMARY AND EXPLANATION

The MicroVue C4a Enzyme Immunoassay is a 96 well, direct-capture immunoassay for the measurement of C4a in human or primate serum, plasma, and other biological or experimental samples. Under normal conditions, activation of the classical or lectin complement pathways results in the cleavage of the complement protein, C4 into C4a and C4b by the protease, C1s. C4a is rapidly cleaved to its more stable, less active form C4a-des Arg form by endogenous serum carboxypeptidase N enzyme. Thus, quantitation of C4a-des Arg should provide a reliable measurement of classical or lectin complement pathway activation that has occurred in test samples.<sup>1-4</sup>

The MicroVue C4a assay, a rapid, highly specific and quantitative procedure for measuring C4a levels, is designed for investigations into the role or status of complement pathway activation in numerous research settings, and for monitoring the generation of C4a *in vivo* or *in vitro*. C4a is the weakest of the anaphylatoxins, compared with C3a and C5a, however, it does play a role in bringing about vascular permeability changes, the induction of smooth muscle contraction and the release of histamine from mast cells and basophils. C4a is believed to play a role in several autoimmune diseases including rheumatoid arthritis, SLE and acute glomerulonephritis.<sup>5-15</sup> It has recently been implicated as a marker for both acute and chronic Lyme disease.<sup>16-17</sup>

## PRINCIPLE OF THE PROCEDURE

The MicroVue C4a Enzyme Immunoassay for the quantitation of C4a is a three-step procedure utilizing (1) a microassay plate coated with a mouse monoclonal antibody that binds specifically to human C4a, (2) an HRP-conjugated monoclonal anti-human C4a antibody, and (3) a chromogenic substrate.

In Step 1, Standards, Controls, and test specimens are added to microassay wells pre-coated with a specific anti-C4a monoclonal antibody. C4a, but not C4 or other complement activation products, present in the specimens will bind to the immobilized anti-C4a monoclonal antibody. After incubation, a wash cycle removes unbound material.

In Step 2, horseradish peroxidase (HRP)-conjugated monoclonal anti-human C4a antibody is added to each test well. The enzyme-conjugated anti-human C4a antibody binds to C4a captured in the microassay wells. After incubation, a wash cycle removes unbound, excess conjugate.

In Step 3, a chromogenic enzyme substrate is added to each microassay well. The bound HRP-conjugate reacts with the substrate, forming a blue color. After incubation, the enzyme reaction is stopped chemically, the color changes to yellow, and the color intensity is measured spectrophotometrically at 450 nm. The color intensity of the reaction mixture is proportional to the concentration of C4a present in the test specimens, Standards, and Controls.

## REAGENTS AND MATERIALS PROVIDED

**96 Assays for the C4a fragment of complement protein, C4**

**MicroVue C4a Enzyme Immunoassay kit contains the following:**

- |                         |  |                    |
|-------------------------|--|--------------------|
| <b>A C4a Standards:</b> | <b>Parts 5204 – 5208</b>   | <b>1.5 mL each</b> |
| <b>B</b>                | Ready to use. Each contains purified native human C4a protein with an assigned protein concentration                 |                    |
| <b>C</b>                | (ng/mL), protein stabilizers   |                    |
| <b>D</b>                |  |                    |
| <b>E</b>                |  |                    |
| <b>L Low Control</b>    | <b>Part 5209</b>   | <b>1.5 mL</b>      |
|                         | Ready to use. Contains purified native human C4a protein with an assigned concentration (ng/mL), protein stabilizers |                    |

<b>H High Control</b>	<b>Part 5210</b>	<b>1.5 mL</b>
Ready to use. Contains purified native human C4a protein with an assigned concentration (ng/mL), protein stabilizers		
<b>1 Microassay Plate</b>	<b>Part 5198</b>	<b>8 wells, 12 each</b>
Eight-well strips coated with a murine monoclonal antibody specific for human C4a in a resealable foil pouch		
<b>2 Stop Solution</b>	<b>Part A9947</b>	<b>12 mL</b>
Contains 1N (4%) Hydrochloric Acid		
<b>3 20X Wash Solution Concentrate</b>	<b>Part A9957</b>	<b>50 mL, 2 each</b>
Contains phosphate buffered saline (PBS), 1.0% Tween-20® and 0.035% Proclin® 300		
<b>4 Complement Specimen Diluent</b>	<b>Part A3670</b>	<b>50 mL</b>
Contains PBS, 0.05% Tween-20, 2.5% protein stabilizers, 0.035% ProClin 300		
<b>5 TMB Substrate</b>	<b>Part 5059</b>	<b>12 mL</b>
Ready to use. Contains 3,3',5,5'-tetramethylbenzidine (TMB) and Hydrogen Peroxide (H <sub>2</sub> O <sub>2</sub> )		
<b>6 Conjugate</b>	<b>Part 5211</b>	<b>12 mL</b>
Contains horseradish peroxidase-conjugated monoclonal anti-human C4a antibody suspended in HRP stabilizing buffer with preservative		

Tween-20® is a registered trademark of ICI Americas Inc.  
ProClin® is a registered trademark of Rohm and Haas Company.

## MATERIALS REQUIRED BUT NOT PROVIDED

- Timer (60 minute range)
- Clean, unused microassay plates, 96 well dilution plate and/or test tubes and racks
- Container for wash buffer dilution
- Wash bottle
- Micropipettes and sterile, disposable pipette tips
- Adjustable multichannel pipette (8 or 12 channels) or repeating micropipettes
- Clean pipettes, 1 mL, 5 mL, and 10 mL
- Reagent reservoirs for adding conjugate, substrate and stop solutions to plate (use clean, unused reservoirs for each reagent)
- Plate reader capable of A<sub>450</sub> 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 Controls 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." <sup>18</sup>
- 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 and contact with metal or rubber 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 6). 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](http://quidel.com).

## STORAGE

Store the unopened kit 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 either of these conditions occur, the solution should be discarded.

## 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

### *Serum/Plasma*

Due to complement activation that occurs during clotting, the C4a concentration in human serum samples will typically be higher than the concentration obtained with EDTA plasma samples. The C4a levels in EDTA plasma may therefore more accurately represent *in vivo* concentrations.

The C4a fragment is susceptible to proteolysis in improperly collected or stored specimens, and C4a may be generated in improperly handled specimens through artifactual complement activation; therefore, the proper collection, storage, and handling of specimens is essential.<sup>19</sup> For optimal plasma results, K2 or K3 EDTA collection tubes are recommended.

Serum and EDTA plasma specimens should be collected aseptically using standard techniques.<sup>20</sup> The specimens should be tested immediately or stored on ice for no longer than four hours before being assayed.

If the specimen cannot be tested within four hours under the guideline detailed above, the specimen should be frozen at  $-70^{\circ}\text{C}$ , or below.

### 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  $37^{\circ}\text{C}$  until just thawed. Transfer thawed specimens immediately to ice to prevent complement activation prior to dilution. Keep specimens on ice for no longer than four hours. Do not leave specimens at  $37^{\circ}\text{C}$ , as complement activation may occur. Do not thaw specimens at room temperature or on ice, as this can lead to complement activation and affect test outcome. Specimens should be tested as soon as possible after thawing. Up to five freeze thaw cycles 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 exceeding the recommended number of 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.**

Specimens must be diluted so that values observed are above the LLOQ and do not exceed the ULOQ. Specimens with readings outside this range should be re-assayed at a new dilution.

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

### *Plasma*

The recommended dilution for plasma samples in Specimen Diluent is 1:40. Perform the dilution as follows:

*10  $\mu\text{L}$  sample + 390  $\mu\text{L}$  Specimen Diluent*

### *Serum*

The recommended dilution for serum samples in Specimen Diluent is 1:80. Perform the dilution as follows:

*10 µL sample + 790 µL Specimen Diluent*

Specimens with high levels of complement activation may require larger sample dilutions than indicated above.

### Add 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 may be used to add diluted specimens, Standards, Controls, and buffer to the wells (see Step 4 of *ASSAY PROCEDURE*). For 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, Quidel recommends using the “replica plating” procedure described below to load the Standards, Controls, and diluted specimens into the microassay wells as rapidly as possible.

Use a multichannel pipettor to 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 being transferred.

**This “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 15°C to 27°C before use.**

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

### Microassay Strips

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

### 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.

### Standards and Controls

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

### ASSAY PROCEDURE

**Read entire product insert before beginning the assay.**

See *WARNINGS AND PRECAUTIONS* and *REAGENT PREPARATION*.

1. Record the microassay well positions corresponding to the blank well(s), all test samples, Standards, and Controls, as well as the indicated lot numbers from the vial labels. Label one corner of the Microassay Plate for orientation.
2. Prepare the microassay strips as follows:
  - a. Rehydrate microassay wells by adding approximately 300  $\mu$ L of Wash Solution to each well using a wash bottle.
  - b. Incubate at 15°C to 27°C for two minutes.
  - c. Remove the liquid from each well.
  - d. Add approximately 300  $\mu$ L Wash Solution to each well.
  - e. Remove the liquid from each well.
  - f. Repeat steps d-e one more time for a total of three washes.**
  - g. Invert the plate and tap firmly on absorbent paper twice to remove any remaining liquid.
3. Select one or more wells to serve as a blank. Add 100  $\mu$ L of Specimen Diluent to the well(s) that will be used to blank the plate reader.
4. Add 100  $\mu$ L Standards, Controls, or diluted specimens to the assigned duplicate wells. The entire plate must be loaded within 15 minutes of loading the first sample onto the plate.
5. Incubate at 15°C to 27°C for 60  $\pm$  5 minutes.
6. Wash the microassay wells a total of 5 times using the following procedure:
  - a. Aspirate the contents from each well.
  - b. Using a wash bottle or automated plate washing device, add approximately 300  $\mu$ L Wash Solution to each well.
  - c. Incubate the wells for 1 minute at 15°C to 27°C.
  - d. Aspirate the contents from each well.
  - e. Invert the plate and tap firmly on absorbent paper to remove any remaining liquid.
  - f. Add approximately 300  $\mu$ L Wash Solution to each well.
  - g. Aspirate the contents from each well.
  - h. Invert the plate and tap firmly on absorbent paper to remove any remaining liquid between each wash.
  - i. Repeat steps f-h four additional times for a total of five washes.**
  - j. After the wash cycle, invert the plate and tap firmly on absorbent paper to remove any remaining liquid.
7. Using a multichannel or repeating pipette, dispense 100  $\mu$ L of C4a Conjugate into each washed test well, including the blank well(s).
8. Incubate the microassay strips at 15°C to 27°C for 60  $\pm$  5 minutes.
9. Wash the microassay wells after the 60-minute incubation (step 8), as described under *ASSAY PROCEDURE*, step 6.
10. Immediately following the wash procedure, dispense 100  $\mu$ L of the TMB Substrate Solution into each well, including the blank(s).
11. Incubate the microassay strips at 15°C to 27°C for 15  $\pm$  1 minutes.
12. Add 100  $\mu$ 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 had been added.
13. Gently tap the plate on the bench top to disperse the color development completely and evenly.  
**NOTE: Optimal results may be obtained by using the plate reader's auto-mix function (if available) just prior to reading the plate.**
14. Determine the absorbance reading at 450 nm for each test well within 30 minutes after the addition of the Stop Solution (step 12), making a blank correction in accordance with the spectrophotometric system in use.
15. Determine the concentration of Samples and Controls from the standard curve.
16. Dispose of the remaining diluted specimens, substrate, and the used microassay strips (*see WARNINGS AND PRECAUTIONS*).

## QUALITY CONTROL

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.

Quality 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.

## INTERPRETATION OF RESULTS

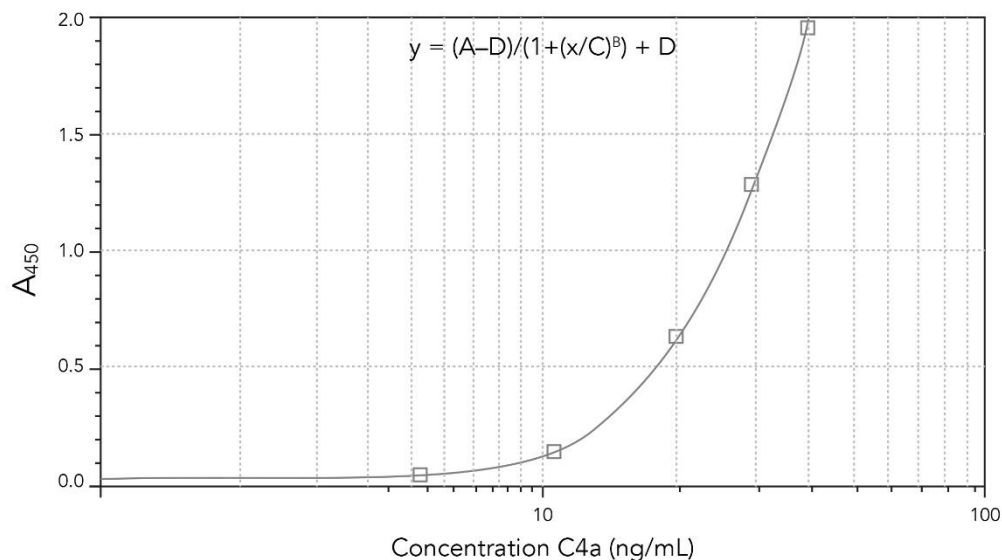
### Use of the Standard Curve

The standard curve for the C4a EIA is generated using the blank-subtracted  $A_{450}$  values for each Standard (on the y axis) and the assigned concentration for each C4a Standard (on the x axis). After 4-parameter regression, the generated standard curve must meet the validation requirements (see below). Most plate reading software and computers are capable of performing these calculations.

Alternatively, the data may be graphed manually and the values (ng/mL) of the test samples read directly from the best-fit line of the standard curve. An example of a typical standard curve is shown in Figure 1.

### Representative Standard Curve

**Figure 1**



Sample	$A_{450}$	ng/ml
Standard A	0.05	5.19
Standard B	0.179	10.51
Standard C	0.657	20.29
Standard D	1.301	29.57
Standard E	1.956	40.34



## Calculation of Actual C4a Concentration in Test Specimens

The actual C4a concentration present in each undiluted test specimen is determined by multiplying the C4a ng/mL concentration, determined from the Kit Standard Curve, by the reciprocal of the specimen dilution factor used.

If the  $A_{450}$  values for a given test specimen are greater than the values of the highest Standard (E), the results should be reported as “greater than” the C4a concentration of the highest Standard (E) multiplied by the specimen dilution factor. If a more accurate C4a concentration value is required, the test specimen should be re-assayed using a larger dilution factor. In all repeat assays, the C4a Standards and Controls must also be run.

## VALIDATION

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correlation coefficient (r):	$\geq 0.98$
blank:	$\leq 0.150$

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Refer to the vial labels or product C of A for the mean acceptable C4a concentration ranges for the High and Low Controls.

## LIMITATIONS OF THE PROCEDURE

The MicroVue C4a Enzyme Immunoassay has been used to test specimens collected as serum, or as plasma in K2 or K3 EDTA. It is not recommended to use Citrated Plasma or Heparin Plasma in the MicroVue C4a assay as they will produce erroneous results. Other anticoagulants have not been tested.

## PERFORMANCE CHARACTERISTICS

### Limits

**LOD:** The limit of detection (LOD) for the C4a EIA is 0.29 ng/mL, determined by the upper 3SD limit in a zero standard study.

**LLOQ:** The lower limit of quantitation (LLOQ) for the C4a EIA is 5.0 ng/mL, the lowest concentration on the standard curve that met CLSI criteria for accuracy and precision.

**ULOQ:** The upper limit of quantitation (ULOQ) for the C4a EIA is 61 ng/mL, the highest concentration that met CLSI criteria for accuracy and precision.

### Interfering Substances

The following substances were tested in the C4a EIA and found to not interfere with the assay using plasma or serum samples:

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Substance	Concentration
Bilirubin	40 mg/dL
Hemoglobin	200 mg/dL
Triglycerides	3000 mg/dL
Glucose	1000 mg/dL
Cholesterol	500 mg/dL
Albumin	6000 mg/dL

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Gamma Globulin	6000 mg/dL
EDTA	10 mM
Heparin	3 U/mL

## Precision

Intra- and inter-assay precision were determined by assaying 20 replicates of 1 plasma sample and 1 serum sample in 10 different assays.

Sample	C4a (ng/mL)	Intra-assay <sup>1</sup> C.V. (%)	Inter-assay <sup>2</sup> C.V. (%)
EDTA Plasma	833.9	3.7%	4.4%
Serum	1941.5	4.3%	4.0%

<sup>1</sup>n = 20 replicates    <sup>2</sup>n = 10 runs

## Linearity

Linearity was performed by diluting samples with specimen diluent and comparing observed values with expected values. Typical results are provided below.

Sample	Dilution Factor	Observed C4a (ng/mL) <sup>3</sup>	Expected C4a (ng/mL) <sup>3</sup>	Recovery (%)
EDTA Plasma	60	35.27	35.27	100%
	80	27.247	26.45	103%
	100	21.678	21.16	102%
	120	18.156	17.64	103%
Serum	80	23.657	23.657	100%
	95	20.331	19.92	102%
	110	17.743	17.21	103%
	125	15.885	15.14	105%

<sup>3</sup>Dilution factor not included.

## Spike Recovery

Spike Recovery was performed by spiking samples with a known quantity of purified C4a and comparing observed values with expected values.

Sample	C4a (ng/mL)	Spike (ng/mL)	Result (ng/mL)	Recovery (%)
Plasma 1	899.598	–	1211.02	92%
Plasma 2	996.199	416.91	1288.55	91%
Plasma 3	902.898	–	1257.25	95%
Serum 1	1000.298	–	1526.67	84%
Serum 2	1522.56	807.258	2090.01	90%
Serum 3	675.658	–	1570.40	106%

## 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](mailto:customerservice@quidel.com) or [technicalsupport@quidel.com](mailto: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](http://quidel.com).

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**REF** A035 – MicroVue C4a Fragment EIA Kit

**RUO**



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**PIA035000EN00 (10/16)**

## GLOSSARY

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**REF**

Catalogue number

**LOT**

Batch code

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Use by



Manufacturer

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Temperature limitation



Consult e-labeling  
instructions for use

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Biological risks

**RUO**

For Research use only

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Contains sufficient for 96 determinations

**CONT**

Contents/Contains

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**CONTROL**

Control

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