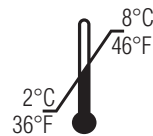


MICROVUE™ iC3b EIA Kit

Complement

An enzyme immunoassay for the
quantitation of the iC3b fragment of C3
protein in human plasma or serum

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



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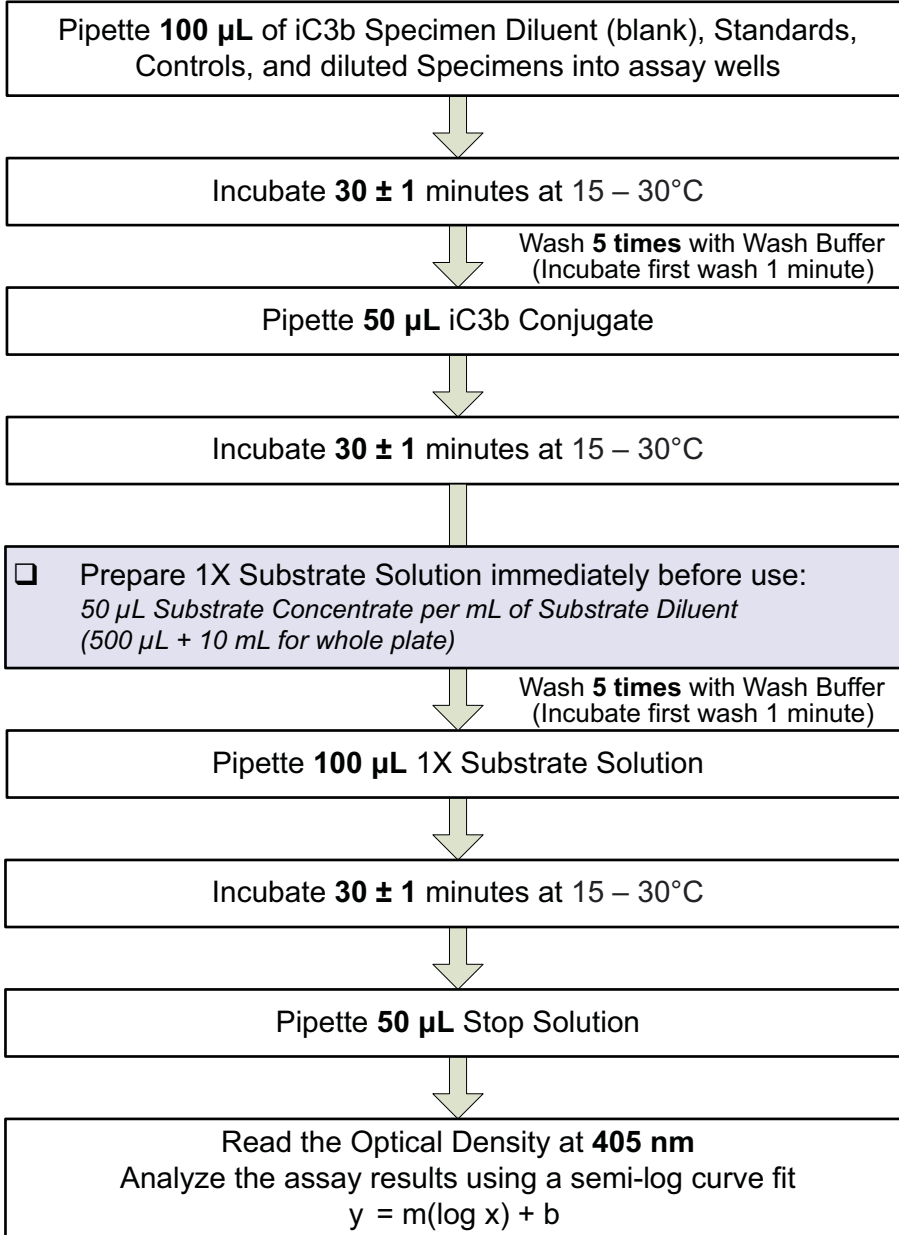
REF A006

MicroVue™ iC3b Enzyme Immunoassay Summary

Reagent, Standards, Controls, and Sample Preparation

- Dilute Wash Buffer Concentrate **1:20** with DI Water
- Reconstitute each Standard and Control with **2.0 mL** of Hydrating Reagent (*let sit for 15 minutes, and mix gently before use*)
- Dilute Specimen Samples between **1:25** and **1:200** in iC3b Specimen Diluent

Assay Procedure



SUMMARY AND EXPLANATION

The MicroVue iC3b Enzyme Immunoassay measures the amount of the iC3b in human plasma or serum, as well as in other biological fluids, experimental samples, or mixtures.

Under normal conditions, complement activation results in the formation of C3 convertases^{1,2} that cleave C3 into two fragments — C3a and C3b. The C3a fragment is one of the complement anaphylatoxins.³ The C3b fragment has many important biological activities,⁴ including the promotion of phagocytosis by opsonization and participation as a structural component in C3 and C5 convertases. These biological activities of C3b are under stringent control *in vivo*. One mechanism limiting the *in vivo* lifetime of C3b involves the two-site cleavage of C3b by Factor I⁵ with the cooperation of Factor H⁶ or CR1⁷ as cofactors. Factor H is a complement control protein. CR1 is the C3b/C4b receptor found on many cell types, including red blood cells, granulocytes, monocytes, and macrophages.⁷ Factor I two-site cleavage of C3b yields inactivated C3b, called iC3b. The biological activities of C3b are lost when it is cleaved by Factor I. iC3b fragments, either in fluid phase or bound to biological surfaces, express new biological activities due to their ability to interact with CR2 and CR3 receptors on a variety of cell types.

The levels of iC3b can be significantly elevated in the serum and plasma of some patients with immune complex-associated diseases such as rheumatoid arthritis and systemic lupus erythematosus.^{8,9} iC3b levels may also be elevated in body fluids from other patients in which complement activation is known to occur, e.g., from patients with infections, burns, myocardial infarctions, glomerulonephritis, and acute respiratory distress syndrome. The correlation between iC3b levels and the clinical status or prognosis for patients with these and other diseases remains to be determined and is a question of potential significance to the biomedical community.

The MicroVue iC3b Enzyme Immunoassay provides a rapid, non-radioactive, highly specific and quantitative procedure for measuring this product of C3 activation in serum, plasma, and other body fluids. It may also be used as a research tool to monitor the generation of iC3b *in vitro*.

PRINCIPLE OF THE PROCEDURE

The MicroVue iC3b Enzyme Immunoassay for the quantitation of iC3b in human serum or plasma is a three-step procedure utilizing (1) a microassay plate coated with monoclonal anti-human iC3b, (2) an HRP-conjugated anti-human iC3b, and (3) a chromogenic substrate.

In the first step, standards, controls, and test specimens are added to microassay wells precoated with an anti-iC3b monoclonal antibody. The anti-iC3b monoclonal antibody is specific for iC3b and will not bind to C3, C3b, nor any other smaller C3b degradation fragment.⁹ iC3b present in the Standards, Controls, or specimens will bind to the immobilized anti-iC3b. After incubation, a wash cycle removes unbound material.

In the second step, horseradish peroxidase (HRP)-conjugated goat anti-human iC3b is added to each test well. In this step, the enzyme conjugated anti-iC3b binds to iC3b that was captured by the monoclonal anti-iC3b on the surface of the microassay wells. After incubation, a wash cycle removes unbound, excess conjugate.

In the third step, a chromogenic enzyme substrate is added to each microassay well. The bound HRP-conjugate reacts with the substrate forming a green color. After incubation the enzyme reaction is stopped chemically, and the color intensity is measured spectrophotometrically at 405 nm. The color intensity of the reaction mixture is proportional to the concentration of iC3b present in the test specimens, Standards, and Controls.

REAGENTS AND MATERIALS PROVIDED

96 Assays for the iC3b fragment of C3 protein

MicroVue iC3b Enzyme Immunoassay contains the following:

| | | |
|---|---------------------------|------------------|
| A iC3b Standards: | Parts A9548-A9550 | 2 mL each |
| B (Lyophilized) When reconstituted, each consists of diluted human serum | | |
| C containing known amounts of iC3b in PBS, Protein Stabilizers, 0.01% Thimerosal | | |
| L Low/High Controls | Parts A9551, A9552 | 2 mL each |
| H (Lyophilized) When reconstituted, each consists of diluted human serum containing iC3b diluted in PBS, Protein Stabilizers, 0.01% Thimerosal | | |
| 1 iC3b Microassay Plate | Part A9546 | 12 each |
| Mouse Anti-Human iC3b monoclonal antibody adsorbed onto eight-well strips in a resealable foil pouch | | |
| 2 Stop Solution | Part A3673 | 6 mL |
| Contains 250mM Oxalic Acid | | |
| 3 20X Wash Solution Concentrate | Part A3674 | 2 x 50 mL |
| Phosphate buffered saline (PBS), 0.05% Tween-20®, and 0.01% Thimerosal | | |
| 4 iC3b Specimen Diluent | Part A9547 | 25 mL |
| Contains PBS, 0.05% Tween-20, Protein Stabilizers, 0.01% Thimerosal | | |
| 5 Substrate Diluent | Part A3672 | 25 mL |
| Contains 0.1M Citrate Buffer and 0.05% H ₂ O ₂ | | |
| 6 Substrate Concentrate | Part A3671 | 1.5 mL |
| Contains 0.7% 2,2'-Azino-bis(3-ethylbenzthiazoline-6-sulfonic acid), diammonium salt | | |
| 7 iC3b Conjugate | Part A9553 | 2 x 3 mL |
| Peroxidase-conjugated (goat) anti-human iC3b in PBS, Stabilizers, 0.01% Thimerosal | | |
| 8 Hydrating Reagent | Part A3675 | 25 mL |
| Contains 0.035% ProClin® 300 | | |

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)
- Calculator or other computational method to validate the assay
- Micropipettes and pipette tips to deliver 10-100 µL
- Adjustable multi-channel pipette (8 or 12 channels) or repeating micropipettes. (Optional)
- Items suitable for liquid measurement of 10-1000 mL
- Container for wash solution dilution
- Wash bottle or other immunoassay washing system
- Deionized or distilled water
- Sodium hypochlorite (household bleach, 1:10 dilution) and/or autoclave for decontamination of specimens, reagents and materials
- Plate reader capable of optical density readings between 0.0 and 2.0
- Semi-log calibration curve fitting software

WARNINGS AND PRECAUTIONS

1. For Research Use Only. Not for use in diagnostic procedures.
2. Treat specimen samples as potentially biohazardous material. Follow Universal Precautions when handling contents of this kit and any patient samples.
3. Wear suitable protective clothing, gloves, and eye/face protection when handling contents of this kit.
4. Use the supplied reagents as an integral unit prior to the expiration date indicated on the package label.
5. Store assay reagents as indicated.
6. Do not use Coated Strips if pouch is punctured.
7. Thimerosal is used as a preservative. Incidental contact with or ingestion of buffers or reagents containing thimerosal can lead to increased hypersensitivity reactions including irritation to the skin, eyes, or mouth. Seek medical attention if symptoms are experienced. Exposure to thimerosal may have potential mutagenic effects. Avoid contact with strong acids and bases.
8. 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.
9. 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," 2007.
10. Proper collection and storage of test specimens are essential for accurate results (see *SPECIMEN COLLECTION AND STORAGE*).
11. Avoid microbial or cross-contamination of specimens or reagents.
12. Do not use a microassay well for more than one test.
13. Using incubation times and temperatures other than those indicated in the Procedure section may give erroneous results.
14. The Substrate Concentrate is light sensitive. Avoid prolonged exposure to bright or direct light. Store reagents in the dark when not in use.
15. Do not allow microassay wells to dry once the assay has begun.
16. When removing liquid from the microassay wells, do not scrape or touch the bottom of the wells.
17. Heat-inactivated, hyperlipemic, or contaminated specimens may give erroneous results.

18. Use only new or clean materials/lab supplies to perform this assay. After running the assay, decontaminate all surfaces by washing with a freshly prepared 1:10 solution of household bleach (sodium hypochlorite) or exposing to a UV light source at 254nm for a minimum of 5 minutes. Decontaminate lab supplies and/or waste specimens, reagents, and materials by soaking for a minimum of 30 minutes in a freshly prepared 1:10 solution of household bleach or autoclave at 121°C for 30 minutes at 15 psi. Alternatively, discard any used supplies/materials in appropriately-marked biohazard waste containers.¹⁰
19. To avoid aerosol formation during washing, use an apparatus to aspirate the wash fluid into a bottle containing household bleach.
20. A wash bottle or automated filling device should be used to wash the plate (*ASSAY PROCEDURE*, Step 7). For best results, do not use a multichannel pipette to wash the microassay plate.
21. Dispose of containers and unused contents in accordance with Federal, State and Local regulations.

REAGENT PREPARATION

Bring all reagents and materials to 15–30°C before use.

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

Refer to Table 1 for the amounts of reagents and materials required per number of wells.

Coated Strips

Determine the number of strips needed for the assay by referring to Table 1. Remove the desired number of strips. Secure the selected strips that are to be used in the plate frame. Place the unneeded strips back into the storage bag and store at 2-8°C.

Wash Solution

Prepare the Wash Solution for washing the micro-assay wells by diluting 50 mL of the 20X Wash Solution Concentrate up to a final volume of one liter with distilled or deionized water. Mix thoroughly before use. The Wash Solution is stable for 30 days when stored in a clean container at 2-8°C. If cloudiness occurs, discard the reagent.

NOTE: If crystals are present in the 20X Wash Solution Concentrate, warm the bottle in a 37-50°C water bath with frequent mixing, until all crystals have been dissolved.

iC3b Standard and Control Reconstitution

Add 2.0 mL of Hydrating Reagent to each Standard vial (A-C) and to the Low Control and the High Control. Allow the reconstituted vials to rehydrate for at least 15 minutes at room temperature. Mix thoroughly. Avoid formation of foam or bubbles during mixing. Reconstituted Standards and Controls are stable for 30 days when stored at 2-8°C.

Specimen Dilution

Caution: Treat all specimens as if potentially infectious.¹¹ Do not use heat-inactivated or contaminated specimens.

Prepare an appropriate dilution of each specimen using the Specimen Diluent (see *CALCULATION OF RESULTS*). Mix thoroughly, but avoid formation of foam or bubbles. Do not store or re-use diluted specimens.

Adding Diluted Specimens to the Microtiter Wells

Either of two methods can be used to add diluted specimens, Standards, Controls, and Buffer, to the wells (see Step 5 of *ASSAY PROCEDURE*). For small assay runs where only a few specimens are being tested, the diluted specimens and other reagents can 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. **(A multi-channel pipettor may be used to conveniently add the Conjugate, Substrate and Stop Solution, as well).**

In order 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, 120-130 µL of each solution can be added 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.

Preparation of Substrate Solution

Prepare just prior to use. Determine the required volume of Substrate Solution from Table 1, below. **Prepare the Substrate Solution by adding 50 µL of Substrate Concentrate to each mL of Substrate Diluent.** Mix thoroughly. Do not prepare the Substrate solution until step 9 of the Assay Procedure. If the substrate solution turns dark green prior to use, discard it, and make a fresh solution in a clean container.

**TABLE 1
REAGENT REQUIREMENTS**

| Wells ¹ | 8-Well Strips | Substrate Solution Volume (mL) |
|--------------------|---------------|--------------------------------|
| 16 | 2 | 2.0 |
| 24 | 3 | 3.0 |
| 32 | 4 | 4.0 |
| 40 | 5 | 5.0 |
| 48 | 6 | 5.0 |
| 56 | 7 | 6.0 |
| 64 | 8 | 7.0 |
| 72 | 9 | 8.0 |
| 80 | 10 | 9.0 |
| 88 | 11 | 9.0 |
| 96 | 12 | 10.0 |

¹Determine the number of specimens to be tested and add eleven (11) wells for the three Standards, the Low and High Controls to be tested (in duplicate) and one blank well. It is recommended that duplicate Standards and Controls be tested in separate microassay strips when possible.

STORAGE

Store the unopened kit at 2-8°C.

All reagents must be brought to room temperature (15-30°C) before use. Place all unused microassay strips into the storage bag, reseal the bag, and store at 2-8°C.

INDICATIONS OF INSTABILITY OR DETERIORATION OF REAGENTS

1. The Substrate Concentrate will range in color from colorless to pale or even dark green. Variation in the color of the Substrate Concentrate is normal and in no way affects the performance of the test. However, the freshly prepared Substrate Solution should be colorless to pale green. A dark green color indicates that the reagent has deteriorated, must be discarded, and new Substrate Solution must be prepared in clean glassware.
2. Cloudiness of the Wash Solution indicates a deterioration of this reagent. If this occurs, the solution should be discarded.

SPECIMEN COLLECTION AND STORAGE

Handle and dispose of all specimens using Universal Precautions.

The proper collection and storage of specimens is essential, since C3 is highly susceptible to proteolysis, e.g., by plasmin, and to water hydrolysis. These events may artificially generate additional iC3b or iC3(H₂O) molecules *in vitro*. iC3(H₂O) is also detected in the assay. Rapid processing of serum or plasma, and proper manipulation and storage of specimens, significantly reduces these modes of C3 consumption.

Serum or EDTA plasma specimens should be collected aseptically using standard techniques. The specimens should be tested immediately or stored at 4°C or on ice until assayed. However, this short-term storage on ice should not exceed four hours.

For longer-term storage, serum or plasma should be separated and frozen at -70°C or below within two hours after collection.

Specimen Stabilizing Solution, [REF] A9576, can also be used to prepare human serum and plasma specimens for storage. Proper use of this solution, which is available only from Quidel, requires that the specimen be mixed 1:1 with the solution before freezing at -70°C. Further technical information about this product is available upon request.

Frozen specimens should be tested as soon as possible after thawing or stored on ice (for no longer than four hours) until assayed.

Normal values for serum samples will be higher than those obtained with EDTA plasma samples. The iC3b levels in EDTA plasma may more accurately represent the *in vivo* concentrations.

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. Select one or more wells to serve as a blank, depending upon the EIA scanner used. Add 100 μ L of iC3b Specimen Diluent to the well(s) that will be used to blank the plate reader.
3. Add 100 μ L of each reconstituted iC3b Standard (A, B, C) to duplicate wells.
4. Add 100 μ L of iC3b High Control (Human) and 100 μ L of iC3b Low Control (Human) to duplicate wells.
5. Add 100 μ L of each diluted specimen to its assigned microassay well (see *REAGENT PREPARATION*).
6. Incubate at room temperature (15-30°C) for 30 \pm 1 minutes.
7. Wash the microassay wells as follows:
 - a. After the incubation in step 6 (or in step 9 below) aspirate the contents from each well.
 - b. Using a wash bottle or automated plate washing device, add approximately 300 μ L Wash Solution to each well.
 - c. Incubate the wells for 1 minute at room temperature (15-30°C).
 - d. Remove the fluid from each well.
 - e. Add approximately 300 μ L Wash Solution to each well.
 - f. Remove the fluid from each well.
 - g. **Repeat steps e-f three additional times.**
 - h. After the fifth wash cycle, invert the plate and tap firmly on absorbent paper to remove any remaining liquid.
8. Using a multichannel or repeating pipette, dispense 50 μ L of iC3b conjugate into each washed test well, including the blank well(s).
9. Incubate the microassay strips at room temperature (15-30°C) for 30 \pm 1 minutes. Prepare the Substrate Solution during this incubation (see *REAGENT PREPARATION*).
10. Wash the microassay wells after the 30-minute incubation (step 9), as described under *ASSAY PROCEDURE*, step 7.
11. Immediately following the wash procedure, dispense 100 μ L of the freshly prepared Substrate Solution into each well, including the blank(s).
12. Incubate the microassay strips at room temperature (15-30°C) for 30 \pm 1 minutes.
13. Add 50 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 had been added.
14. Gently tap the plate to disperse the color completely and evenly.
15. Determine the absorbance reading at 405 nm for each test well within one hour after the addition of the Stop Solution (step 13), making a blank correction in accordance with the spectrophotometric system in use. A reference filter is not required for this assay.
16. Dispose of the remaining diluted specimens, controls, 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. The optical density values are provided and are to be used as a guideline only. The results obtained by your laboratory may differ.

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

Calculation of Results

The optimum dilution of patient samples or other serum or plasma research specimens may vary under some circumstances, e.g., due to differences in sample collection, processing, or storage. For this reason, it is recommended that the user determine the optimum specimen dilution under controlled specimen collection procedure(s) defined by his/her experimental approach. To determine this dilution, several normal subjects (3-5 individuals) may be sampled and tested. The optimum dilution is one that yields A_{405} values for the normal or low control specimens between 0.10 and 0.30. The kit has been optimized so that specimen dilutions between 1:25 and 1:200 in Specimen Diluent are usually acceptable. Both EDTA and heparin anti-coagulated plasma and serum can be used in this kit.

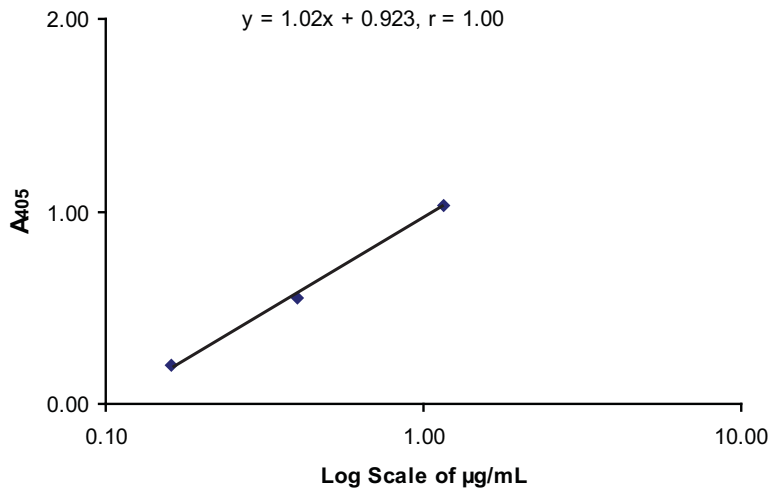
Use of the Standard Curve

The A_{405} value for each iC3b Standard (y) and the assigned μg of iC3b per mL ($\mu\text{g}/\text{mL}$) value for each Standard (x) may be used to establish a standard curve. The μg of iC3b/mL for iC3b standards A-C are indicated on the vial labels. The best-fit line is drawn on semi-log graph paper.

An alternative to this graphic method is to derive the best-fit line using various automated methods. The logarithm (base 10) of the concentration of each of the three iC3b standards (x) must be entered to assure linearity. The corresponding A_{405} values (y) are entered without change. Most computers and calculators are capable of performing these calculations.

On graph paper, the $\mu\text{g}/\text{mL}$ concentration for each diluted sample may be read on the x axis for each corresponding A_{405} value obtained. If the calculator method is used, the A_{405} value is entered and the corresponding logarithm (base 10) concentration (x value) is determined. The iC3b concentration of the diluted sample is the anti-log of this value. An example of a typical semi-log curve is shown in Figure 1.

FIGURE 1



Calculation of Actual iC3b Concentration in Patient or Research Specimens

The assigned concentration on the standard vials and the control vials are absolute units of iC3b protein. The concentration of iC3b in a patient or research sample is determined by multiplying the determined concentration by the reciprocal of the specimen dilution made. For example, if a normal patient specimen yields an absorbance of 0.20 units, the linear regression curve may yield a concentration of 0.2 µg iC3b/mL. If the sample was diluted 1:50 for the assay, the concentration of iC3b in the patient specimen would be 10 µg iC3b/mL or (0.2 x 50). If the Quidel **Specimen Stabilizing Solution**, [REF] A9576, is being used, one must account for the added specimen dilution required for the proper use of this solution (see Technical Information Sheet for this product).

Validation

Determine the slope, intercept, and correlation coefficient of the derived best-fit line for the iC3b A, B, and C Standards. The values must be within the specified ranges to qualify the assay:

- correlation coefficient (r): > 0.95
- slope (m): between 0.72 and 1.30
- y-intercept (b): between 0.70 and 1.39

Refer to the vial labels for the iC3b concentration ranges of the High and Low Controls.

LIMITATIONS

The performance characteristics for this assay have not yet been established in various patient populations. **This kit is for research use only and is not intended for use in diagnostic procedures.**

The MicroVue iC3b Enzyme Immunoassay has been used to test specimens collected as serum or as plasma in EDTA and heparin anticoagulant. Other anticoagulants have not been tested.

ASSISTANCE

To place an order or for technical assistance, please contact a Quidel Representative at 800-524-6318 or 408-616-4301, Monday through Friday, between 8:00 a.m. and 5:00 p.m., Pacific Time. Orders may also be placed by fax at 408-616-4310.

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

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Manufacturer



Catalog Number



Consult Instructions for Use



Temperature Limitation



Contents / Contains



Instructions for Use on CDROM



Contains sufficient for <n> tests



Biological Risks

REF A006 – MicroVue™ iC3b Enzyme Immunoassay Kit