QUIDEL

MicroVue™ Complement Factor I EIA

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

Reagent, Standards, Controls, and Sample Preparation

- ☐ Dilute Wash Solution Concentrate 1:20 with DI Water
- ☐ Specimen Preparation (1:1500)
  - ☐ Dilution 1: Dilute Specimen 1:100 with Complement Specimen Diluent *(5 µL Specimen + 495 µL Complement Specimen Diluent)*
  - ☐ Dilution 2: Dilute Specimen 1:15 from Dilution 1 with Complement Specimen Diluent *(25 µL from Dilution 1 + 350 µL Complement Specimen Diluent)*

Assay Procedure

Pipette ~**300 µL** of wash solution into assay wells

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Aspirate liquid from each well (Blot dry)

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Pipette **100 µL** of Complement Speciment Diluent (blank), Standards, Controls, and Samples into assay wells

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Incubate **60 ± 1 minutes** at 22˚C to 28˚C

Wash **4 times** with Wash Buffer

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Pipette **100 µL** Conjugate

↓

Incubate **60 ± 1 minutes** at 22˚C to 28˚C

Wash **4 times** with Wash Buffer

↓

Pipette **100 µL** Substrate Solution

↓

Incubate **15 ± 1 minutes** at 22˚C to 28˚C

↓

Pipette **100 µL** Stop Solution

↓

Read the Optical Density at **450 nm**
Analyze the assay results using quadratic regression
$y = A+Bx+Cx^2$

INTENDED USE

The MicroVue Factor I EIA is an enzyme immunoassay for the quantitative measurement of complement Factor I.

SUMMARY AND EXPLANATION

The complement system is a part of the innate immune response.[1] It can be activated via three initiation pathways: Classical Pathway, Lectin Pathway, and Alternative Pathway.[2] The complement system consists of numerous proteins located in the blood as inactive precursors. Complement activation leads to a cascade of interactions where specific proteins are cleaved, with the ultimate end goal being the recruitment of phagocytes and the creation of the Membrane Attack Complex (MAC), which is a complex of complement proteins that creates pores within cell membranes, leading to cell lysis and death.[3-5]

The Classical/Lectin pathways lead to protein cleavage fragment C4b, while all three pathways lead to cleavage fragment C3b. Cleavage fragments C4b and C3b are critical for sustained complement response. Complement Factor I protein is a negative regulatory protein and controls the complement system by cleaving and inactivating cell-bound or fluid phase C3b and C4b.[6] Importantly, Factor I must be in the presence of a co-factor to function. These co-factors include C4BP, CR1, Factor I, or MCP.

Factor I is a soluble glycoprotein found in human blood at an average concentration of 35 µg/mL.[7] It is a heterodimer consisting of a heavy chain and light chain via a disulfide link. Factor I is primarily synthesized in the liver.[8] However, it may also be expressed by endothelial, keratinocytes, monocytes, and other cell types.

Factor I has been implicated in the research of a variety of autoimmune diseases as its loss of function leads to increased complement activity. This includes Atypical Hemolytic Uremic Syndrome (aHUS), Age-Related Macular Degeneration (AMD), and among others.[9, 10]

PRINCIPLE OF THE PROCEDURE

MicroVue Factor I EIA is a three-step procedure utilizing (1) a microassay plate coated with a mouse monoclonal antibody that binds specifically to human Factor I, (2) an HRP-conjugated murine anti-human Factor I, and (3) a chromogenic substrate.

In the first step, Standards, Controls, and test specimens are added to microassay wells precoated with a specific anti-Factor I monoclonal antibody. Factor I, but not other complement activation products, present in the Standards, Controls, and specimens will bind to the immobilized anti-Factor I monoclonal antibody. After incubation, a wash cycle removes unbound material.

In the second step, horseradish peroxidase (HRP)-conjugated murine anti-Factor I antibody is added to each test well. The enzyme conjugated anti-Factor I binds to Factor I captured in 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 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 Factor I present in the test specimens, Standards, and Controls.

REAGENTS AND MATERIALS PROVIDED

96 Assays for Factor I

MicroVue Factor I EIA kit contains the following:

A **Factor I Standards:** **Parts 1349400-1349800** **1 mL each**

B Each contains a known concentration of Factor I in human serum diluted in PBS, protein stabilizers,0.05% Tween-

C 20, 0.035% ProClin® 300

D

E

L **Factor I Low Control** **Part 1349000** **1 mL**

Each contains a known concentration of Factor I in human serum diluted in PBS, protein stabilizers,0.05% Tween-20, 0.035% ProClin® 300

H **Factor I High Control** **Part 1348900** **1 mL**

Each contains a known concentration of Factor I in human serum diluted in PBS, protein stabilizers,0.05% Tween-20, 0.035% ProClin® 300

❶ **Microassay Plate** **Part 1349200** **12 x 8 wells**

12 eight-well strips coated with a purified mouse monoclonal antibody specific for human Factor I in a resealable foil pouch

❷ **Stop Solution** **Part A9947** **12 mL**

Contains 1N Hydrochloric acid

❸ **20X Wash Solution Concentrate** **Part A9957** **50 mL**

Contains phosphate buffered saline (PBS), 1.0% Tween-20®, and 0.035% ProClin 300

❹ **Complement Specimen Diluent** **Part A3670** **1 x 50 mL**

Each contains PBS, 0.05% Tween-20, 2.5% protein stabilizers, 0.035% ProClin 300

❺ **TMB Substrate** **Part 5059** **12 mL**

Contains 3,3', 5,5' tetramethylbenzidine (TMB) and hydrogen peroxide (H_2O_2)

❻ **Factor I Conjugate** **Part 1348800** **12 mL**

Contains horseradish peroxidase-conjugated murine anti-human Factor I 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)
- Calculator or other computational method to validate the assay
- Clean, unused microassay plates and/or test tubes and racks
- Container for wash buffer dilution
- Wash bottle
- Adjustable multichannel pipette (8 or 12 channels) or repeating micropipettes (optional)
- Clean pipettes, 1 mL, 5 mL, and 10 mL
- Micropipettes and pipette tips
- Plate reader capable of optical density readings between 0.0 and 3.0
- Deionized or distilled water
- Spectrophotometer capable of reading at 450 nm

WARNINGS AND PRECAUTIONS

- Treat specimen samples as potentially bio hazardous 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.
- When adding or removing liquids from the microassay wells, do not scrape or touch the bottom of the wells.
- Do not allow microassay wells to dry once the assay has begun.
- Do not use a microassay well for more than one test.
- 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. Pipet carefully using only calibrated equipment.
- Proper collection and storage of test specimens are essential for accurate results (see *SPECIMEN COLLECTION AND PREPARATION*, page 6).
- Avoid microbial or cross-contamination of specimens, reagents, or materials. Incorrect results may be obtained if contaminated.
- 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 Substrate is light sensitive. Avoid prolonged exposure to bright or direct light. Store reagents in the dark when not in use.
- A wash bottle should be used to wash the plate (ASSAY PROCEDURE, Step 5). For best results, do not use a multichannel pipette to wash microassay plates.
- 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.

REAGENT PREPARATION

Bring all reagents and materials to 15°C to 25°C before use.
After removing the needed reagents and materials, return the unused items to their appropriate storage temperatures (see *STORAGE*).

Coated Strips

Determine the number of wells needed for the assay. Remove the desired number of strips necessary to meet the desired number of wells. Secure the selected strips that are to be used in the plate frame. Place the unneeded strips back into the storage bag, seal the bag, and store at 2°C to 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 (1) 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°C to 8°C. If cloudiness occurs, discard the reagent.

Specimen Dilution

Caution: Treat all biological specimens as if potentially infectious.

It is recommended that, if using human serum or plasma specimens, they be diluted 1:1500 in Specimen Diluent for use in the MicroVue Factor I EIA.

- Dilution 1: Dilute Specimen 1:100 with Complement Specimen Diluent (5 µL Specimen + 495 µL Complement Specimen Diluent)
- Dilution 2: dilute Specimen 1:15 from dilution 1 with Complement Specimen Diluent (25 µL Dilution 1 + 350 µL Complement Specimen Diluent)

Adding Diluted Specimens to the Microtiter Wells

Either of two (2) methods can be used to add diluted specimens, Standards, Controls, and Buffer, to the wells (see Step 3 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, Quidel recommends 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 µL to130 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.

STORAGE

Store the unopened kit at 2°C to 8°C. After the kit is opened, the 20X Wash Solution Concentrate may be stored at 2°C to 25°C.

All reagents, including specimens, must be brought to room temperature (15°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 of the Wash Solution indicates a deterioration of this reagent. If this occurs, the solution should be discarded.

SPECIMEN COLLECTION AND PREPARATION

Handle and dispose of all specimens using Universal Precautions.

The proper collection and storage of specimens is essential.

Samples collected in Sodium Citrate tubes generated results that were approximately 14% lower than matched serum or EDTA samples and are not recommended for this assay. Samples collected in Lithium Heparin and Sodium Heparin demonstrated slightly elevated replicate variability.

Serum or EDTA plasma specimens should be collected aseptically using standard techniques. The specimens should be tested immediately, stored at 4°C, or on ice for no longer than four (4) hours before being assayed.

If the specimen cannot be tested within four (4) hours under the guidelines detailed above, the specimen should be frozen at –70°C, or below.

Thaw frozen (≤–70°C) specimens rapidly in a 37°C water bath until just thawed. Transfer thawed specimens immediately to ice (for no longer than four hours) to prevent complement activation prior to dilution. **Do not leave specimens at 37°C.** Do not thaw specimens at room temperature or 4°C, as this can lead to complement activation. Frozen specimens should be tested as soon as possible after thawing. Repeated freezing and thawing is not recommended. If samples are to be re-frozen for further analysis, we suggest freezing multiple aliquots of the specimen to prevent repeated freeze/thaw cycles.

RECOMMENDED 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. Using a wash bottle or automated plate washing device, add approximately 300 µL Wash Solution to each well.
   b. Aspirate the contents from each well.
   c. Invert the plate and tap firmly on absorbent paper to remove any remaining liquid.
3. Add 100 µL of Specimen Diluent (blank), Standards, Controls, or 1:1500 diluted specimens to the assigned wells.
4. Incubate at 22°C to 28°C for 60 ± 1 minutes.
5. Wash the microassay wells a total of 4 times using the following procedure:
   a. Remove the contents from each well.
   b. Using a wash bottle or automated plate washing device, add approximately 300 µL Wash Solution to each well.
   c. Remove the contents from each well. (Blot dry)
   d. Add approximately 300 µL Wash Solution to each well.
   e. Remove the contents from each well.
   f. Repeat steps d and e two (2) additional times.
   g. After the fourth wash cycle, invert the plate and tap firmly on absorbent paper to remove any remaining liquid. (Blot dry)
6. Using a multichannel or repeating pipette, dispense 100 µL of Factor I Conjugate into each washed test well, including the blank well(s).
7. Incubate the microassay strips at 22°C to 28°C for 60 ± 1 minutes.
8. Wash the microassay wells after the 60-minute incubation (step 7), as described under *ASSAY PROCEDURE*, step 5.
9. Immediately following the wash procedure, dispense 100 µL of the TMB Substrate Solution into each well, including the blank(s).
10. Incubate the microassay strips at 22°C to 28°C for 15 ± 1 minutes.
11. Add 100 µL 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.
12. Gently tap the plate on the bench top to disperse the color development completely and evenly.

13. Determine the absorbance reading at 450 nm for each test well within 40 minutes after the addition of the Stop Solution (step 11), making a blank correction in accordance with the spectrophotometric system in use.
14. Analyze the assay results using a quadratic regression curve equation $y=A+Bx+Cx^2$.
15. 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 in the generation of a standard curve.

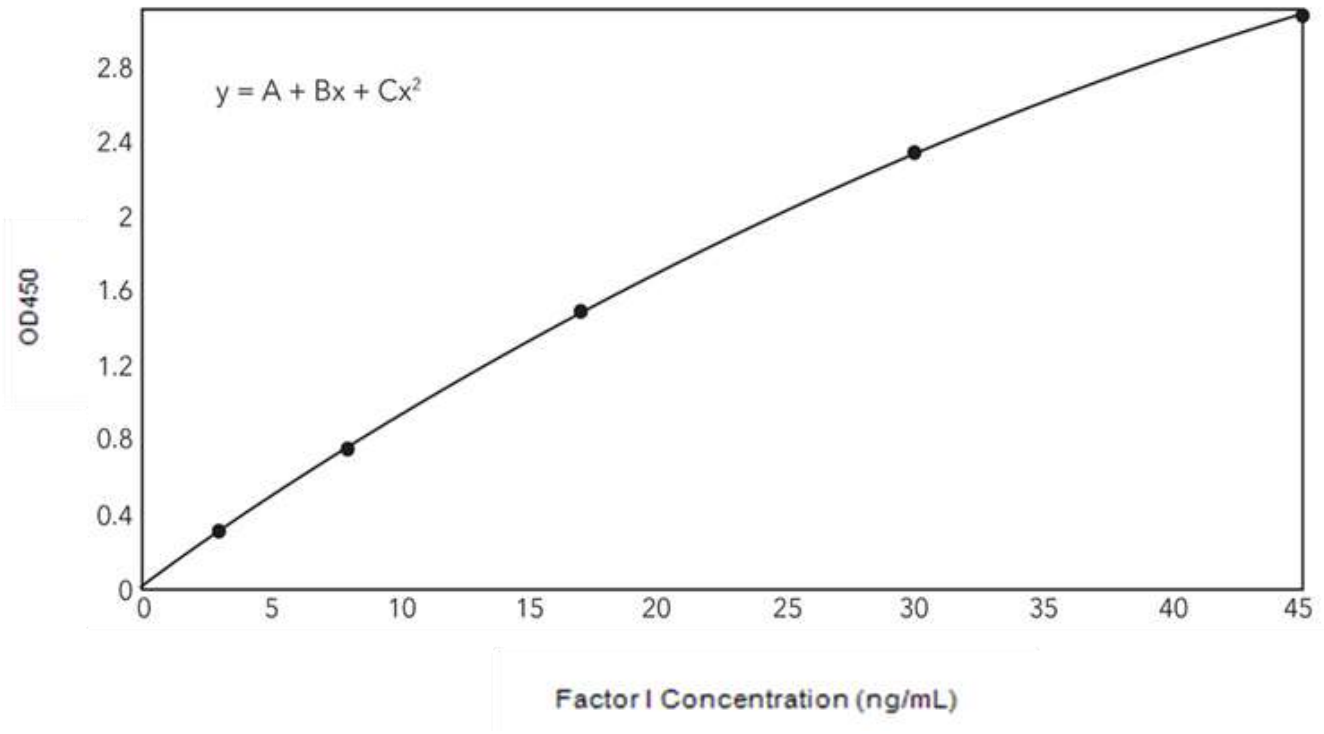
Kit control ranges are provided. The control values are intended to verify the validity of the curve and sample results. Each laboratory should establish its own parameters for acceptable assay limits. If the control values are NOT within your laboratory's acceptance limits, the assay results should be considered questionable, and the samples should be repeated.

CALCULATION OF RESULTS

The Standard curve for the MicroVue Factor I EIA is generated using the blank-subtracted A_{450} values for each Standard (on the y axis) and the assigned concentration for each Factor I Standard (on the x axis). Most plate reading software and computers are capable of performing these calculations.

Alternatively, the data may be graphed manually. An example of a typical standard curve is shown in figure 1.

Figure 1: Example of Standard Curve



Calculation of Actual Factor I Concentration in Test Specimens

The Factor I concentration present in each undiluted test specimen is determined by multiplying the Factor I/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 that of the highest Standard (E), the results should be reported as "greater than" the Factor I concentration of the highest Standard (E) multiplied by the specimen dilution factor.

LIMITATIONS

The MicroVue Factor I EIA has been used to test specimens collected as serum or EDTA plasma.

PERFORMANCE OF THE TEST

Limits

LOD: The limit of detection (LOD) for the Factor I EIA is 0.5 ng/mL.

LLOQ: The lower limit of quantitation (LLOQ) for the Factor I EIA is 1.6ng/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 Factor I EIA is 82.1 ng/mL, the highest concentration that met CLSI criteria for accuracy and precision.

Interfering Substances

The following substances were tested in the Factor I EIA and found to not interfere or cross-react with the assay:

Substance	Passing Concentration	
Bilirubin	0.8	mg/mL
Hemoglobin	5.0	mg/mL
Triglycerides	30	mg/mL
Albumin	60	mg/mL
Glucose	6	mg/mL
Gamma Globulin	60	mg/mL
BSA	120	mg/mL
Cholesterol	5.0	mg/mL
Lithium Heparin 1	15.8	USP units/mL
Lithium Heparin 2	31.6	USP units/mL
Sodium Citrate 1	0.02	M
Sodium Citrate 2	0.04	M
Potassium Oxalate 1	2	mg/mL
Potassium Oxalate 2	4	mg/mL

Precision

Within-run and between-run precision was determined by assaying 20 replicates of two (2) plasma samples and two (2) serum samples in 11 different runs.

Sample	Factor I (µg/mL)	Between-run[1] C.V. (%)	Within-run [2] C.V. (%)
EDTA Plasma	40.0	5.8	2.9
	12.3	5.7	3.0
Serum	20.7	5.8	2.8
	9.1	4.4	3.0

[1]n = 11 runs

[2]n = 20 replicates

Linearity

Linearity was performed by serially diluting samples prior to testing and comparing observed values with expected values. Typical results are provided below.

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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A041 – MicroVue Factor I EIA

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PIA041001EN00 (02/19)

GLOSSARY

REF Catalogue number	LOT Batch code
 Use by	 Manufacturer
 Temperature limitation	 Consult e-labeling instructions for use
 Biological risks	RUO For Research use only
 Contains sufficient for 96 determinations	CONT Contents/Contains
CONTROL Control	