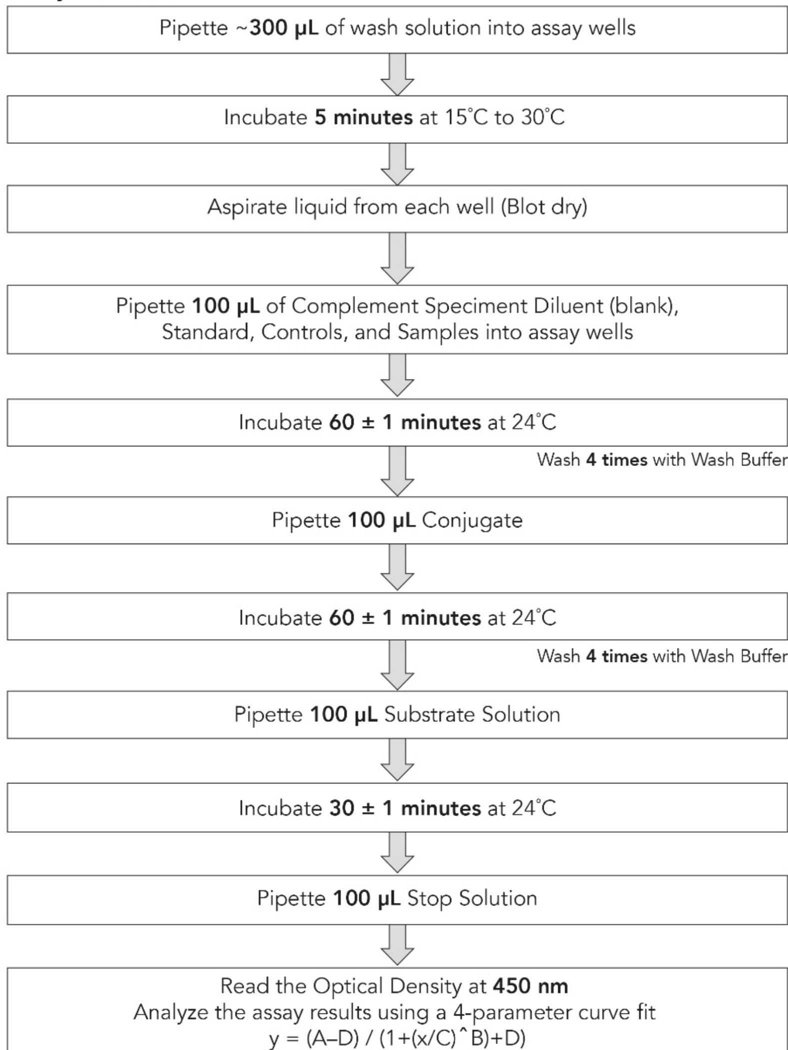
MicroVue™ Complement
Factor H EIA

An enzyme immunoassay for the quantitative measurement of complement Factor H in human plasma or serum.

SUMMARY



INTENDED USE
The MicroVue Factor H EIA is an enzyme immunoassay for the quantitative measurement of the complement Factor H in human plasma or serum.

SUMMARY AND EXPLANATION
The alternative complement pathway provides innate protection against microbial agents in the absence of specific antibody.[1-5] The activation of this complement pathway can be triggered by a variety of substances including microbial polysaccharides or lipids, gram negative bacterial lipopolysaccharides, and surface determinants present on some viruses, parasites, virally infected mammalian cells, and cancer cells. In autoimmune diseases, the alternative complement pathway may contribute directly to tissue damage.
Factor H is involved in the regulation of the alternative pathway of complement. In blood, activation of C3, under normal conditions, is kept at a low level by control proteins, Factor H and Factor I. Factor H functions in two ways to inactivate the C3bBb enzyme: 1) it accelerates the dissociation of Bb from C3b; and 2) serves as a cofactor for Factor I, a serine protease, which cleaves C3b into iC3b, which can no longer form the C3 convertase with Factor B.[6]

Factor H also regulates the spontaneous fluid-phase activation of the alternative complement pathway by C3b-like forms of C3 that continuously arise in plasma and serum. Therefore, when concentrations of Factor H fall below normal levels, there is rapid fluid-phase activation and consumption of complement components both *in vivo* and *in vitro*.[9]

Factor H is a single-chain glycoprotein with a molecular weight of 150 KD.[7] Concentrations found in normal human plasma/serum is approximately 500 µg/mL, although it can range from 116-562 µg/mL depending on multiple factors (environment and genetic).[7] Factor H regulates complement activation on the cellular surface and in fluid phase while participating in roles for both the alternative and classical pathways.

The majority of Factor H is produced in the liver. However, it can also be expressed locally by endothelial cells, epithelial cells, platelets, mesenchymal stem cells, and among others.[7]

Known levels of Factor H aid in the diagnosis of several disease states such as atypical Hemolytic-Uremic Syndrome (aHUS), age-related macular degeneration, and dense deposit disease. Complement Factor H has been implicated in the research of many autoimmune diseases. Studies have included using Factor H as a serum biomarker of multiple sclerosis disease state, as a therapy for renal diseases associated with Factor H abnormalities, and as a camouflage to tumor cells for protection against the host immune system. This broad range of testing gives Factor H an appeal to many types of research.

PRINCIPLE OF THE PROCEDURE
MicroVue Factor H EIA is a three-step procedure utilizing (1) a microassay plate coated with a mouse monoclonal antibody that binds specifically to human Factor H, (2) an HRP-conjugated murine anti-human Factor H, 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 H monoclonal antibody. Factor H, but not other complement activation products, present in the Standards, Controls, or specimens will bind to the immobilized anti-Factor H monoclonal antibody. After incubation, a wash cycle removes unbound material.

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

REAGENTS AND MATERIALS PROVIDED

96 Assays for Factor H

MicroVue Factor H EIA kit contains the following:

A B C D E **Factor H Standards:** **Parts A9848 through A9852** **1 mL each**
Each contains a known concentration of Factor H in human serum diluted in PBS, protein stabilizers,0.05% Tween-20, 0.035% ProClin® 300

L **Factor H Low Controls** **Part A9853** **1 mL**
Each contains a known concentration of Factor H in human serum diluted in PBS, protein stabilizers,0.05% Tween-20, 0.035% ProClin® 300

H **Factor H High Controls** **Part A9854** **1 mL**
Each contains a known concentration of Factor H in human serum diluted in PBS, protein stabilizers,0.05% Tween-20, 0.035% ProClin® 300

❶ **Microassay Plate** **Part A9560** **12 x 8 wells**
12 eight-well strips coated with a purified mouse monoclonal antibody specific for human Factor H 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**
Each contains phosphate buffered saline (PBS), 1.0% Tween-20®, and 0.035% ProClin 300

❹ **Complement Specimen Diluent** **Part A3670** **2 x 50 mL**
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 H Conjugate** **Part A9855** **12 mL**
Contains horseradish peroxidase-conjugated murine anti-human Factor H 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

- For *in vitro* diagnostic use.
- Treat specimen samples as potentially biohazardous material. Follow Universal Precautions when handling contents of this kit and any patient samples.
- Wear suitable protective clothing, gloves, and eye/face protection when handling contents of this kit.
- Dispose of containers and unused contents in accordance with Federal, State and Local regulations.
- 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.
- 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 1:5000 in Specimen Diluent for use in the MicroVue Factor H EIA.

- Dilution 1: Dilute Specimen 1:100 with Complement Specimen Diluent (10 µL Specimen + 990 µL Complement Specimen Diluent)
- Dilution 2: dilute Specimen 1:50 from dilution 1 with Complement Specimen Diluent (10 µL Dilution 1 + 490 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, 120-130 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.

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 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.

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.

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. Incubate at 15°C to 30°C for five (5) minutes.
    c. Aspirate the contents from each well.
    d. 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 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 H 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 30 ± 1 minutes.
11. Add 100 µL of Stop Solution to each well to stop the enzymatic reaction. 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. Dispose of the remaining diluted specimens, controls, substrate, and 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

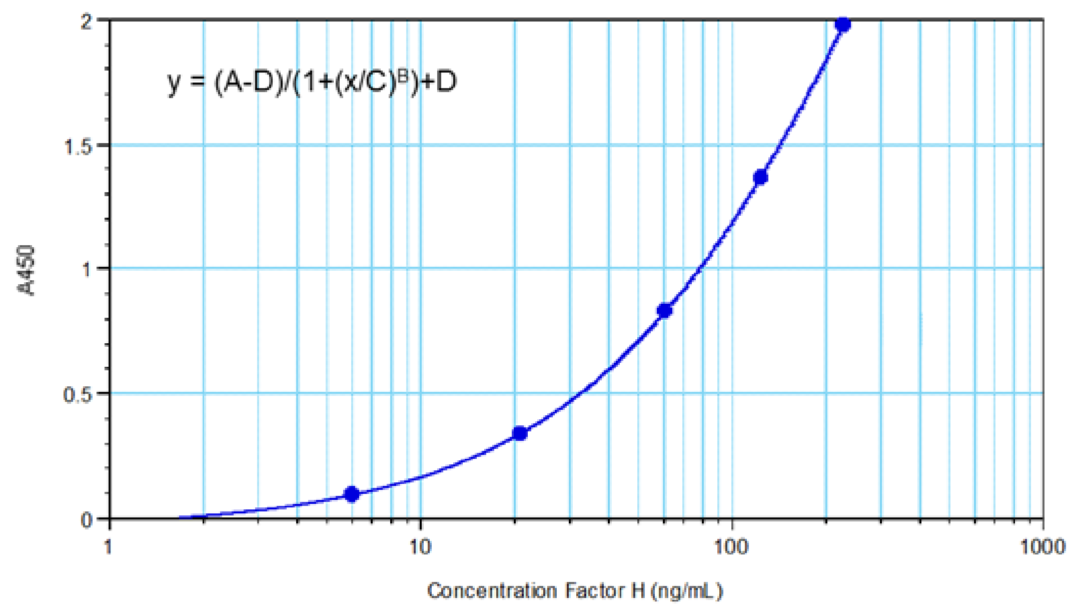
Kit control ranges are provided. The control values are intended to verify the validity of the curve and sample results. Each laboratory should establish its own parameters for acceptable assay limits. If the control values are NOT within your laboratory's acceptance limits, the assay results should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

CALCULATION OF RESULTS

The Standard curve for the MicroVue Factor H EIA is generated using the blank-subtracted A_{450} values for each Standard (on the y axis) and the assigned concentration for each Factor H 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 Standard Curve



Calculation of Actual Factor H Concentration in Test Specimens

The Factor H concentration present in each undiluted test specimen is determined by multiplying the Factor H/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 H concentration of the highest Standard (E) multiplied by the specimen dilution factor.

LIMITATIONS

The MicroVue Factor H 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 H EIA is 3.155 ng/mL.

LLOQ: The lower limit of quantitation (LLOQ) for the Factor H EIA is 4.64 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 Factor H EIA is 521 ng/mL, the highest concentration that met CLSI criteria for accuracy and precision.

Interfering Substances

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

Substance	Concentration
Bilirubin	0.4 mg/mL
Hemoglobin	5.0 mg/mL
Triglycerides	30 mg/mL
Albumin	60 mg/mL

Glucose	12	mg/mL
Gamma Globulin	60	mg/mL
BSA	120	mg/mL
Cholesterol	5.0	mg/mL

Precision

Within-run and between-run precision was determined by assaying 18 replicates of two (2) plasma samples and two (2) serum samples in 10 different runs.

Sample	Factor H (µg/mL)	Within-run[1] C.V. (%)	Between-run[2] C.V. (%)
EDTA Plasma	217	4.1	9.3
	368	4.7	9.4
Serum	96	5.2	9.0
	357	4.8	9.7

[1]n = 20 replicates [2]n = 10 runs

Linearity

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

Sample	Dilution Factor	Observed Factor H (µg/mL)	Expected Factor H (µg/mL)	Recovery (%)
Plasma	Neat	243	243	100.0
	1:8	253	280	90.36
	1:4	317	314	101.1
	1:2.37	331	351	94.44
	1:2	384	355	108.3
	1:1.6	390	393	99.24
	1:1.33	402	425	94.59
	1:1.4	452	434	104.1
	Neat	466	466	100.0
Serum	Neat	12	12	100.0
	1:8	49	56	88.29
	1:4	99	104	95.65
	1:2.37	148	147	100.6
	1:2	195	207	94.20
	1:1.6	257	240	107.0
	1:1.33	285	299	95.48
	1:1.4	354	344	103.0
	Neat	402	402	100.0

SAMPLE VALUES

EDTA plasma and serum from sixty-six (66) donors were tested in the MicroVue Factor H EIA kit. These were paired normal samples with no additional clinical information provided. The results are presented below.

			RANGE	
	n	mean	±2 SD	±3 SD
EDTA Plasma	66	313 µg/mL	196 to 431 µg/mL	138 to 489 µg/mL

Serum	66	309 µg/mL	175 to 443 µg/mL	108 to 510 µg/mL

Note: The mean and Standard Deviation (SD) behavior of Factor H concentrations determined for plasma or serum samples may vary between laboratories. Therefore, it is recommended that each laboratory determine the mean Factor H concentration and standard deviation values for samples

An additional four (4) clinically low samples were obtained that were previously tested using radial immunodiffusion (RID) then tested using the MicroVue Factor H EIA kit. The results using the MicroVue Factor H EIA kit showed comparable results with the RID testing.

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 A040 – MicroVue Factor H EIA

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Revision Changes:

- Added a procedural step (Assay Procedure, 2.b.) requiring a five (5) minute incubation period during the pre-wash activity.

GLOSSARY

REF

Catalogue number

CE mark of conformity

EC REP

Authorized Representative
in the European Community

LOT

Batch code

Use by

Manufacturer

Temperature limitation

Intended use

Consult e-labeling
instructions for use

IVD

For *In Vitro* diagnostic use

Contains sufficient for 96 determinations

CONT

Contents/Contains

CONTROL

Control