



QUIDEL

MicroVue™ Complement CH50 Eq EIA

An enzyme immunoassay to measure the total classical complement pathway activity in human serum

Rx ONLY

SUMMARY

Reagents, Standards, Controls, and Samples Preparation

- Dilute Wash Solution Concentrate 1:20 with deionized water
- Reconstitute each Control with 100 μL deionized or distilled water (let sit for 15 minutes at room temperature. Vortex, then place on ice)

Assay Procedure

Continuously mix Activator by swirling during use

Pipette **86 μL** of Activator to dilution tubes or dilution wells

Pipette **14 μL** of Controls and Specimen into dilution tubes or dilution wells and mix carefully

Cover dilution tubes or dilution wells and incubate **60 \pm 1 minutes** at 37°C (store up to 14 days at $\leq -20^\circ\text{C}$)

- Dilute activated Controls and Specimens 1:200 with Complement Specimen Diluent into new tubes or dilution wells
NOTE: The recommended dilution schema is 5 μL activated sample or control + 995 μL Specimen Diluent

Add **~300 μL** of Wash Solution into assay wells

Incubate **2 minutes** at 20°C to 27°C

Remove liquid from each well (Blot dry)

Pipette **100 μL** of Specimen Diluent (blank), Standards, activated Controls, and activated Specimens into assay wells

Incubate **60 \pm 1 minutes** at 20°C to 27°C

Wash **7 times** with Wash Buffer (Incubate first wash 1 minute)

Pipette **50 μL** of Conjugate

Incubate **60 \pm 1 minutes** at 20°C to 27°C

Wash **7 times** with Wash Buffer (Incubate first wash 1 minute)

Pipette **100 μL** of Substrate Solution

Incubate **15 \pm 1 minutes** at 20°C to 27°C

Pipette **100 μL** of Stop Solution

Read the Optical Density at **450 nm**
Analyze the assay results using a linear curve fit ($y = mx + b$)



INTENDED USE

The MicroVue CH50 Eq EIA measures the total classical complement pathway activity in human serum and allows detection of a deficiency of one or more of the complement components C1 through C9.

SUMMARY AND EXPLANATION

The binding of C1q component of C1 to immune complexes triggers the classical complement pathway. This activation results in a cascade of enzymatic and non-enzymatic reactions, culminating in the formation of terminal complement complexes (TCC). Under standard conditions the level of TCC that can be generated in a serum is a quantitative expression of the serum's total classical complement activity.

The traditional method for measuring the total classical complement activity in serum is the CH50 test.¹ This test is a lytic assay, which uses antibody-sensitized sheep erythrocytes (EA) as the activator of the classical complement pathway and various dilutions of the test serum to determine the amount required to give 50% lysis. The percent hemolysis is determined spectrophotometrically. The CH50 test is an indirect measure of TCC, since the TCC themselves are directly responsible for the hemolysis that is measured.

The MicroVue CH50 Eq EIA provides a direct measure of the total classical complement activity in serum by quantifying the amount of TCC generated under standard conditions. The MicroVue CH50 Eq EIA uses a monoclonal antibody to a unique neoantigen to capture the TCC analyte. Since both the CH50 Eq EIA and the CH50 test rely on the generation of TCC and correlate, the CH50 Eq EIA's results are expressed in CH50 unit equivalents per milliliter.

PRINCIPLE OF THE PROCEDURE

The MicroVue CH50 Eq EIA for quantifying the total classical complement activity in human serum involves three basic procedures: (1) complement activation; (2) sample dilution; and (3) assay for terminal complement complexes (TCC).

To activate the classical complement pathway, undiluted human serum samples and the Controls are added to microassay wells or to test tubes containing the Activator. During incubation the classical pathway of complement is triggered and TCC are generated.

In the second step, the activated sera are diluted in microassay wells or test tubes and dispensed, together with kit Standards, directly into a microassay plate. The TCC present in the activated samples bind to the monoclonal antibodies coating the surface of the microassay wells.

In the third step, the TCC microassay plate is washed and loaded with an HRP-conjugate, which will bind to the bound TCC. After washing, the TCC microplate is loaded with a chromogenic enzyme Substrate. After incubation a reagent is added to stop color development. The absorbencies (A_{450} values) generated with the Controls, kit Standards, and test specimens are measured spectrophotometrically. The color intensity of the reaction mixture is proportional to the concentration of TCC present and to CH50 units. Using the kit standard curve, assay results are expressed in CH50 unit equivalents per milliliter (CH50 U Eq/mL).

REAGENTS AND MATERIALS PROVIDED

The CH50 Eq EIA kit contains the following:

A Standards	Parts A3719-A3723	1 each, 1.5 mL
B	Each contains human serum with assigned CH50 unit equivalents per mL (U Eq/mL), protein stabilizers	
C		
D		
E		
N Normal Controls	Part A3724	3 each, 100 µL
	(Lyophilized) When reconstituted, each contains human serum	
L Low Controls	Part A3725	3 each, 100 µL
	(Lyophilized) When reconstituted, each contains human serum	
1 Microassay Plate	Part A3840	1 each
	96-well with retainer and holder consisting of 12 eight-well strips coated with a mouse monoclonal antibody in a resealable foil pouch	
2 Stop Solution	Part A9947	12 mL
	Contains 1N (4%) Hydrochloric Acid	
3 20X Wash Solution Concentrate	Part A9957	2 each, 50 mL
	Contains phosphate buffered saline (PBS), 1.0% Tween-20® and 0.035% Proclin® 300	
4 Complement Specimen Diluent	Part A3670	50 mL
	Contains PBS, 0.05% Tween-20, 2.5% protein stabilizers, 0.035% ProClin 300	
5 TMB Substrate	Part 5059	12 mL
	Ready to use. Contains 3,3',5,5'-tetramethylbenzidine (TMB) and Hydrogen Peroxide (H ₂ O ₂)	
6 Conjugate	Part A3726	7 mL
	Contains horseradish peroxidase-conjugated (goat) antibodies to TCC	
7 Activator	Part A3718	10 mL
	Contains human gammaglobulins and murine monoclonal antibodies in phosphate buffered saline (PBS) with 0.02% Sodium Azide	

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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 or other immunoassay washing system
- 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 A450 readings between 0.0 and 3.0
- Deionized or distilled water
- Incubator or microassay plate heater (37°C)
- Water bath (37°C) water

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.²
- Wear suitable protective clothing, gloves, and eye/face protection when handling contents of this kit.
- Use the supplied reagents as an integral unit prior to the expiration date indicated on the package label.
- Store assay reagents as indicated.
- Do not use Coated Strips if pouch is punctured.
- ProClin® 300 is used as a preservative. Incidental contact with or ingestion of buffers or reagents containing ProClin can cause irritation to the skin, eyes or mouth. Use good laboratory practices to reduce exposure. Seek medical attention if symptoms are experienced.
- The Stop Solution is considered corrosive and can cause irritation. Do not ingest. Avoid contact with eyes, skin, and clothing. If contact is made, immediately rinse affected area with water. If ingested, call a physician.
- Each donor unit used in the preparation of the standards and control sera of this product was tested by an FDA-approved method for the presence of antibody to human immunodeficiency virus (HIV1 and HIV2) and to hepatitis C virus, as well as for hepatitis B surface antigen. Since no test method can offer complete assurance that infectious agents are absent, these reagents should be handled at Biosafety Level 2 as recommended for any potentially infectious human serum or blood specimen in the Centers for Disease Control/National Institutes of Health manual "Biosafety in Microbiological and Biomedical Laboratories," 2007.
- 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 Collection And Storage*).
- Avoid microbial or cross-contamination of specimens, reagents, or materials. Incorrect results may be obtained if contaminated.
- Test each sample in duplicate.
- Do not use a microassay well for more than one test.
- Using incubation times and temperatures other than those indicated in the *Assay Procedure* section may give erroneous results.
- The TMB Substrate must be protected from light during storage and incubation. Avoid contact with eyes, skin, and clothing. If contact is made, immediately rinse affected area with water.
- Do not allow microassay wells to dry once the assay has begun.
- When adding or aspirating liquids 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 or automated filling device should be used to wash the plate (*Assay Procedure*, Step 9). For best results, do not use a multichannel pipette to wash the microassay plate.
- Dispose of containers and unused contents in accordance with Federal, State and Local regulations.

STORAGE

Store unopened kit at 2°C to 8°C After the kit is opened, the 20X Wash Solution Concentrate may be store at 2°C to 30°C.

After selecting the reagents or materials to be used in the assay, return the unused reagents immediately to their appropriate storage temperatures. Bring reagents and materials to room temperature (20°C to 27°C) before use.

INDICATIONS OF INSTABILITY OR DETERIORATION OF REAGENTS

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

The Activator may contain particles. This is normal and does not affect performance of the assay.

SPECIMEN HANDLING AND PREPARATION

Use serum as the specimen in this assay. Plasma is not acceptable.

Handle and dispose of all specimens using Universal Precautions.

The proper collection, processing, storage, and shipment of specimens is essential, since complement may be activated in improperly handled specimens. This could lead to erroneous results.

Serum specimens should be collected aseptically and prepared using standard techniques for clinical laboratory testing. Samples can be stored up to 2 hours at room temperature, up to 4 hours on ice, up to 3 days at 4°C, and at –70°C or below for long-term storage. Do not subject samples to more than 6 freeze/thaw cycles. Frozen specimens should be tested as soon as possible after thawing or stored on ice (for no longer than 4 hours) until assayed.

Specimens should be packed in excess dry ice for shipping. Specimens that arrive thawed at the testing facility may be compromised. After receiving a shipment, samples can be stored at –70°C or below.

REAGENT PREPARATION

After removing the needed reagents and materials, return the unused items to their appropriate storage temperatures (see *STORAGE*). Bring all reagents and materials for the assay to room temperature (20°C to 27°C) before use.

1. 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.

2. Selecting the Microassay Strips

Determine the number of wells required for the assay. It is recommended that the blank wells, Controls, and Standards be tested in duplicate. Remove the strip retainer from the assembled plate. Remove the unneeded strips and place them in the storage bag, reseal the bag and return it to storage at 2°C to 8°C. Secure the strips to be used in the assay.

3. Specimen Dilution

CAUTION: Treat all specimens as if potentially infectious. Do not use heat-inactivated, contaminated

specimens, or improperly stored specimens. Serum specimens are required for this assay.

Serum samples should be thawed quickly by placing them briefly in a 37°C water bath. Immediately upon thawing, place the samples on ice until the activation step (see *Assay Procedure*). Do not dilute the test samples prior to activation.

4. Preparation of Normal and Low Controls

To one vial each of the lyophilized Normal and Low Control add 100 µL of deionized or distilled water. Mix briefly to ensure reconstitution and let sit for 15 minutes at room temperature. Vortex again and place on ice until ready for the activation step.

ASSAY PROCEDURE

Read entire product insert before beginning the assay.

See *REAGENT PREPARATION* and *WARNING AND PRECAUTIONS* before proceeding.

- 1. Activation of Samples and Controls.** Mix the Activator, which contains readily suspendable particles, by swirling the bottle before use. Swirl frequently during this step to ensure suspension of the particles. Add 86 µL of Activator to the appropriate number of test tubes or microassay wells.* Next add 14 µL of undiluted serum specimen, Normal Control, or Low Control to individual test tubes or microassay wells containing the Activator. Mix carefully. Cover to minimize evaporation. Incubate at 37°C for 60 minutes. (**FOR YOUR CONVENIENCE:** If you wish to batch samples, after activation you may store the undiluted, activated samples at –20°C or colder for up to 14 days before proceeding to the next steps.)
* The number of test tubes or microassay wells required for activation equals the number of test samples plus two (2) additional ones for activation of the two Controls.
- 2. Dilution of Activated Samples and Controls.** After activation, dilute the activated samples and activated Controls 1:200 in Specimen Diluent in clean, unused microassay wells or in test tubes.
NOTE: The recommended dilution schema is 5 µL activated sample or control + 995 µL Specimen Diluent.
- Prepare the microassay strips as follows:
 - Rehydrate microassay wells by filling each with Wash Solution (250-300 µL/well) to each well using a wash bottle or an automated device.
 - Incubate at room temperature (20°C to 27°C) for 2 minutes.
 - Remove the liquid from each well.
 - Invert the plate and tap firmly on absorbent paper repeatedly to remove any remaining liquid.
- Add 100 µL of Specimen Diluent to the duplicate wells that will be used to blank the plate reader.
- Add 100 µL of each ready-to-use Standard (A-E) to duplicate wells. **Note that the standards have already been diluted and are ready to use.**
- Add 100 µL of the diluted, activated Normal and Low Controls to duplicate wells.
- Add 100 µL of each diluted specimen in duplicate to its assigned microassay wells.
- Incubate at room temperature (20°C to 27°C) for 60 ± 1 minutes.
- Wash the microassay wells as follows: Note: *A multi-channel pipette is not recommended for this step.*
 - After the incubation in step 8 (or in step 12 below) remove the liquid from each well.
 - Using a wash bottle, automated plate washer, or other washing device, fill each well with the Wash Solution (250-300 µL/well).
 - Incubate the wells for 1 minute at room temperature (20°C to 27°C).
 - Remove the liquid from each well.
 - Fill each well with Wash Solution (250-300 µL/well).
 - Remove the liquid from each well.
 - Repeat steps e-f five additional times.**
 - After the seventh wash cycle, invert the plate and tap firmly on absorbent paper repeatedly to remove any remaining fluid.

10. Using a multichannel or repeating pipette, dispense 50 μL of Conjugate into each washed test well, including the blank well(s).
11. Incubate the microassay strips at room temperature (20°C to 27°C) for 60 ± 1 minutes.
12. Wash the microassay wells after the 60-minute incubation, as described in step 9.
13. Immediately following the wash procedure, dispense 100 μL of the TMB Substrate Solution into each well, including the blank(s).
14. Incubate the microassay strips at room temperature (20°C to 27°C) for 15 ± 1 minutes.
15. 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 was the Substrate Solution. Gently tap the plate to disperse the color development evenly.
16. Determine the absorbance reading at 450 nm (A_{450} value) for each test well within 1 hour after the addition of the Stop Solution, making the necessary blank correction. No reference filter is required.
17. Dispose of the remaining diluted specimens, activator, controls, and the used microassay strips in accordance with Federal, State and Local regulations. Keep the strip holder and strip retainer for future use.

QUALITY CONTROL

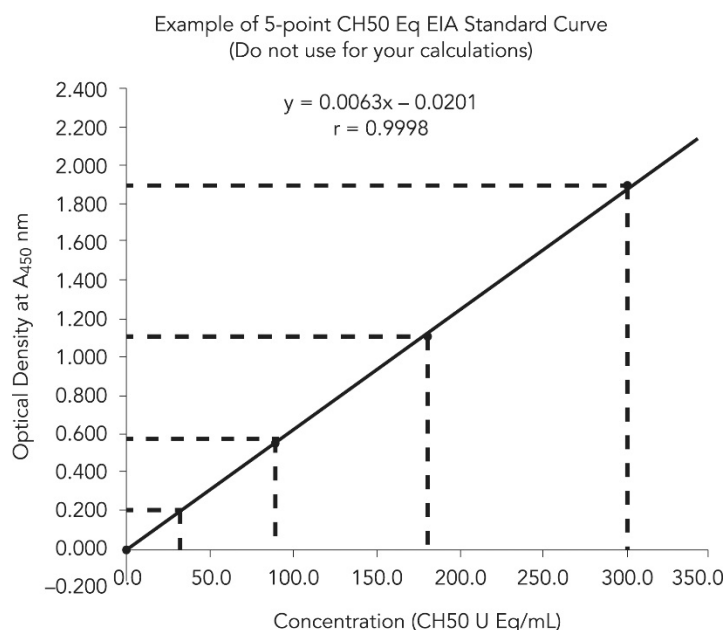
Good laboratory practice recommends the use of controls to ensure that the assay is performing properly. Each CH50 Eq EIA kit contains Normal and Low Controls that can be used for this purpose. These Controls should be tested at least once for each batch of specimens, i.e., for each separate activation run. The Controls, when used as instructed, should give CH50 unit equivalent values within the ranges specified on their vial labels. Since these Controls are to be activated, diluted, and tested exactly like a typical specimen, they serve as both Controls and Reference Standards for each activation and CH50 Eq EIA run. External controls, prepared by your laboratory, may also be used to help ensure that the assay is performing properly.

In addition, the product insert requires that the standard curve generated with the kit A-E Standards meet stringent validation requirements (see *INTERPRETATION OF RESULTS*). Standards should be tested in duplicate for each assay run. If the assay does not meet these requirements, repeat the assay or contact Quidel Technical Assistance.

INTERPRETATION OF RESULTS

Use of the Standard Curve: The standard curve for the CH50 Eq EIA is generated using the blank-subtracted A_{450} values for each standard (on the y axis) and the assigned concentration for each standard (along the x axis). The standard curve must meet the validation requirements. Most computers and calculators are capable of performing this calculation. An example of a typical standard curve is shown in Figure 1.

Figure 1
Example of Standard Curve



Calculation of Actual CH50 U Eq Level in Patient Specimens: Sample values are calculated from the standard curve using linear regression analysis. For test samples that were activated and diluted 1:200 prior to testing, the CH50 U Eq/mL can be read directly from the standard curve. The values (U Eq/mL) for the Normal and Low Controls can also be read directly from the standard curve, since you have also diluted them 1:200.

In order to obtain accurate CH50 determinations for test specimens that yield A₄₅₀ values greater than that of the CH50 Eq EIA Standard E (or that yield A₄₅₀ values less than that obtained with Standard A), the test specimens may be re-assayed at a different dilution so that their new A₄₅₀ values will be within these limits. In all repeat assays the Standards and Controls must also be tested.

If a sample is run in the assay at a dilution other than 200-fold, the CH50 level of the sample is determined by correcting for the difference between the dilution examined and the usual 200-fold dilution. For example, if a sample is diluted 100-fold rather than the usual 200-fold, and the linear regression curve yields a concentration of 100 CH50 U Eq/ml, then the actual CH50 U Eq/mL in the sample is 50 (i.e., 100 CH50 U Eq/mL divided by 2).

Validation

Calculate the CH50 U Eq/mL for the Normal and Low Controls. Also determine the correlation coefficient (r) and the y-intercept (b) of the derived best fit linear curve for the values obtained with the A-E Standards. To validate the assay, the r, b, and Control values must be within the ranges shown below:

Correlation coefficient (r):	> 0.96
y-intercept (b):	(-) 0.090 t (+) 0.068
Slope (m):	0.0033 – 0.0089
Normal & Low Control values:	Within ranges indicated on their respective vial labels

LIMITATIONS OF THE PROCEDURE

The MicroVue CH50 Eq EIA has been used to test specimens collected as serum. The clinical performance of plasma samples prepared with different anticoagulants has not been determined.

EXPECTED VALUES

Two hundred thirty-four (234) normal and abnormal serum samples were tested at Quidel in the CH50 Eq EIA. The average value obtained with the normal sample population was 133 ± 54 CH50 U Eq/mL. As a guideline, the cutoff obtained using Receiver Operator Characteristics (ROC) analysis³ with these normal and abnormal serum specimens was 70 CH50 U Eq/mL. Each laboratory should define its own normal ranges.

Using the MicroVue CH50 Eq EIA, two hundred twenty-one (221) patient samples were tested at a major clinical reference laboratory in the United States. Employing the cutoff of 70 CH50 Eq U/mL and ROC analysis, the overall accuracy of the CH50 Eq EIA, when compared to the combined results from three hemolytic and one EIA-based assay, was greater than 97%.

Using traditional measurements of clinical performance, the MicroVue CH50 Eq EIA also gave a clinical accuracy of greater than 97% with a sensitivity of 93.2% and a specificity of 99.4%. Using the Bablok Passing Algorithm method,⁴ the CH50 U Eq values obtained with the MicroVue CH50 Eq EIA correlated closely with the CH50 values obtained by the traditional hemolytic assay reported by the clinic laboratory, giving a level of significance of $p < 0.001$.

PERFORMANCE CHARACTERISTICS

Precision:

Within-run and between-run precision was determined by assaying 16 replicates of 4 serum samples in 10 different runs.

Sample	CH50 U Eq/mL	Within-run ¹ C.V. (%)	Between- run ² C.V. (%)
Serum	150.4	3.2	6.0
	59.96	4.5	5.4
	98.84	3.6	8.7
	48.86	3.9	6.2

¹n = 16 replicates ²n = 10 runs

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.

REFERENCES

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IVD



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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

Rx ONLY

Prescription use only



Consult e-labeling
instructions for use



Biological risks

IVD

For *In Vitro* diagnostic use



Contains sufficient for 96 determinations

CONT

Contents/Contains

CONTROL

Control
