



MicroVue™ Complement

SC5b-9 Plus EIA

An enzyme immunoassay for the quantitation of the SC5b-9 complex present in human plasma or serum

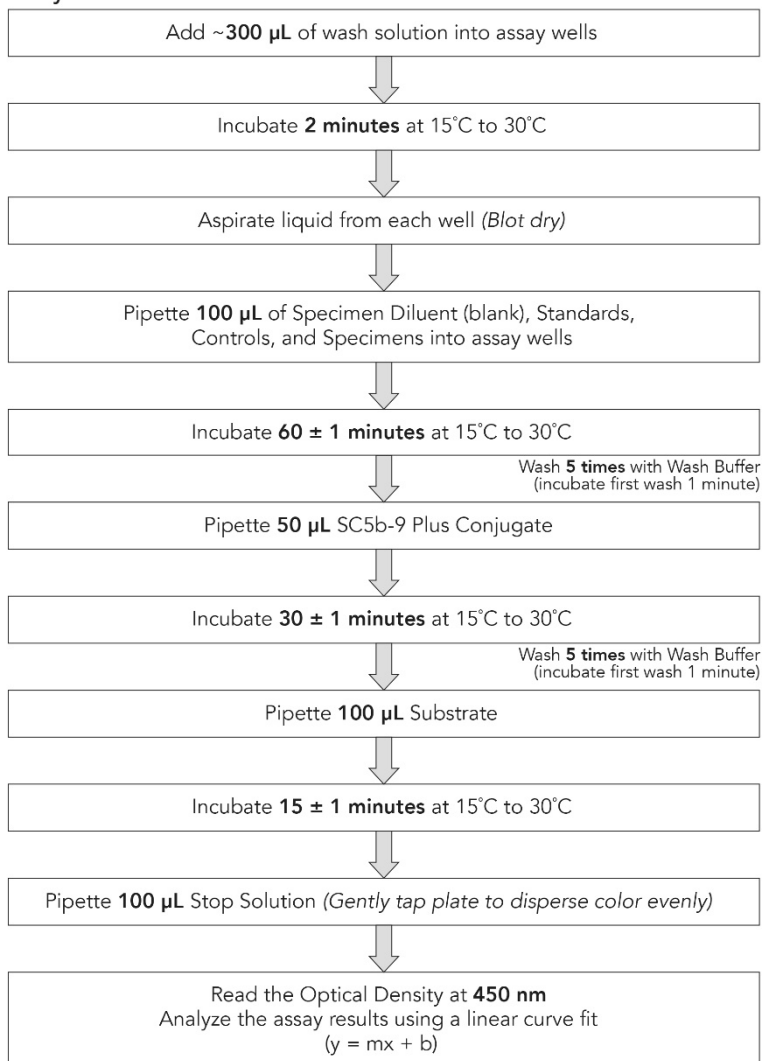
For *In Vitro* Diagnostic Use. For export only. Not for sale in the United States or Canada.

SUMMARY

Reagents and Sample Preparation

- ☐ Dilute Wash Buffer Concentrate 1:20 with DI Water.
- ☐ Dilute Serum Samples 1:40 with Specimen Diluent (e.g. 10 μ L + 390 μ L).
- ☐ Dilute Plasma Samples 1:10 with Specimen Diluent (e.g. 50 μ L + 450 μ L).

Assay Procedure





INTENDED USE

The MicroVue SC5b-9 Plus Enzyme Immunoassay measures the amount of the SC5b-9 complex present in human plasma or serum specimens.

SUMMARY AND EXPLANATION

The Terminal Complement Complex (TCC, SC5b-9) is generated by the assembly of C5 through C9 as a consequence of activation of the complement system by either the classical, lectin or alternative pathway.¹ The membrane attack complex (MAC), a form of TCC, is a stable complex that mediates the irreversible target cell membrane damage associated with complement activation.¹⁻⁴ Complexes formed in the absence of a target membrane bind to naturally occurring regulatory serum proteins, e.g. the S protein,⁵⁻⁷ at the C5b-7 stage of assembly forming soluble, non-lytic TCC.^{1,5} For purposes of this document, we refer to all forms of stable Terminal Complement Complex interchangeably as TCC and SC5b-9, recognizing that other complement regulatory proteins, like Clusterin, also form these stable complexes and are detectable in the SC5b-9 Plus assay.

The MicroVue SC5b-9 Plus Enzyme Immunoassay measures the concentration of TCC thereby giving an indication of the status of the terminal complement pathway in the specimen. It uses a monoclonal antibody to the C9 ring of TCC to capture the complex. The trapped TCC is subsequently detected with HRP-conjugated antibodies that bind to antigens of the SC5b-9 complex. This test, which provides a rapid, highly specific and quantitative procedure for measuring TCC levels, is designed for investigations studying the role or status of terminal complement pathway activation in numerous research settings, and for monitoring the generation of SC5b-9 complexes *in vivo* or *in vitro*. Levels of TCC are indicative of the level of complement activation in the sample. High levels of complement activation have been demonstrated in a variety of disease states including Systemic Lupus Erythematosus (SLE) and other autoimmune diseases, rheumatoid arthritis, acute respiratory distress, and during inflammatory diseases like myocardial infarct and stroke.

PRINCIPLE OF THE PROCEDURE

The MicroVue SC5b-9 Plus Enzyme Immunoassay for the quantitation of SC5b-9 in human serum, plasma, or experimental samples is a three-step procedure utilizing (1) a microassay plate coated with a mouse monoclonal antibody that binds specifically to the C9 ring of SC5b-9, (2) HRP-conjugated antibodies to antigens of SC5b-9, and (3) a chromogenic substrate.

In the first step, standards, controls, and test specimens are added to microassay wells precoated with an anti-SC5b-9 specific monoclonal antibody. SC5b-9 present in the standards, controls, or specimens will bind to the immobilized anti-SC5b-9. After incubation, a wash cycle removes unbound material. Constituent proteins of the TCC, including C9, do not bind to this antibody and are washed away during the wash cycle.

In the second step, horseradish peroxidase (HRP)-conjugated antibodies to antigens on SC5b-9 are added to each test well. The enzyme-conjugated antibodies bind to SC5b-9 that was captured by the monoclonal anti-SC5b-9 bound on the surface of the microassay wells. After incubation, a wash cycle removes unbound 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, a reagent is added to stop color development, resulting in a yellow color. The standard, control, and test specimen absorbances (A_{450} values) are measured spectrophotometrically. The color intensity of the reaction mixture is proportional to the concentration of SC5b-9 (TCC) present in the test specimens, standards, and controls.

REAGENTS AND MATERIALS PROVIDED

96 Assays for SC5b-9 complex

MicroVue SC5b-9 Plus EIA kit contains the following:

A	SC5b-9 Standards:	Parts A9958-62	1.5 mL, 5 each
B	Contains human serum containing known amounts of SC5b-9 in PBS, Protein Stabilizers, Preservatives		
C			
D			
E			
H	High/Low Controls	Parts A9581, A9582	1.5 mL, 2 each
L	Contains human plasma with a high/low level of SC5b-9 complexes, Preservatives		
1	Coated Strips	Part A3840	12 each
	Eight-well strips coated with a mouse monoclonal antibody specific for human SC5b-9 in a resealable foil pouch		
2	Stop Solution	Part 4978	12 mL
	Contains 2N H ₂ SO ₄		
3	20X Wash Solution Concentrate	Part A9957	50 mL, 2 each
	Contains phosphate buffered saline (PBS), 0.05% Tween-20®, and Proclin® 300		
4	Specimen Diluent	Part A3670	50 mL
	Contains PBS, 0.05% Tween-20 Protein Stabilizers, 0.035% Proclin 300		
5	TMB Substrate	Part A9946	12 mL
	Ready to Use peroxide substrate and 3,3',5,5'-tetramethylbenzidine (TMB)		
6	SC5b-9 Plus Conjugate	Part A9577	7 mL
	Contains Horseradish Peroxidase-conjugated (Goat) antibodies to antigens of SC5b-9		

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 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 readings between 0.0 and 2.0
- Deionized or distilled water
- While not required, a plate reader with auto-mix capability is recommended.

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.
- 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 for this product assay is 2N H₂SO₄. Avoid contact with eyes, skin, and clothing. If contact is made, immediately rinse affected area with water.
- 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.
- Proper collection and storage of test specimens are essential for accurate results (see *SPECIMEN COLLECTION AND STORAGE*).
- Avoid microbial or cross-contamination of specimens or reagents.
- Do not use a microassay well for more than one test.
- Decontaminate all specimens, reagents, and materials by soaking for a minimum of 30 minutes in a 1:10 solution of household bleach (sodium hypochlorite) or autoclave at 121°C for 30 minutes at 15 psi.
- Using incubation times and temperatures other than those indicated in the Procedure section may give erroneous results.
- **The TMB Substrate must be protected from light during storage and incubation.** Avoid contact with eyes, skin, and clothing. If contact is made, immediately rinse affected area with water.
- Do not allow microassay wells to dry once the assay has begun.
- When removing liquid from the microassay wells, do not scrape or touch the bottom of the wells.
- Heat-inactivated specimens may yield erroneous results.
- 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 8). For best results, do not use a multichannel pipette to wash the microassay plate.**
- Testing should be performed in an area with adequate ventilation.
- Dispose of containers and unused contents in accordance with Federal, State and Local regulatory requirements.
- Wear suitable protective clothing, gloves, and eye/face protection when handling the contents of this kit.
- Wash hands thoroughly after handling.
- For additional information on hazard symbols, safety, handling and disposal of the components within this kit, please refer to the Safety Data Sheet (SDS) located at quidel.com

STORAGE

Store the unopened kit at 2°C to 8°C. Equilibrate reagents and materials to 15°C to 30°C before use. Place all unused microassay strips into the storage bag, reseal the bag, and store at 2°C to 8°C.

SPECIMEN COLLECTION AND STORAGE

Handle and dispose of all specimens using Universal Precautions.

The proper collection, processing and storage of specimens is essential since SC5b-9 may be generated in improperly handled specimens through artifactual complement activation.

Values for normal serum samples will typically be higher than those obtained with EDTA or citrated normal plasma samples. The SC5b-9 levels in EDTA or citrated plasma may therefore more accurately represent the *in vivo* concentrations.

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

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

A **Specimen Stabilizing Solution** (Part A9576) can also be used to prepare human serum and plasma specimens for storage. Proper use of this product, available only from Quidel, requires that the specimen be mixed 1:1 with the solution prior to freezing. Additional technical information about the solution is available upon request.

Thaw frozen specimens rapidly at 37°C 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, as complement activation may occur. Do not thaw specimens at room temperature or 4°C as this can lead to complement activation. 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, Quidel suggests freezing multiple aliquots of the specimen to prevent multiple freeze/thaw cycles.

REAGENT PREPARATION

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

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

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

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.

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 in the assay plate frame.

Specimen Dilution

Caution: Treat all specimens as potentially hazardous. Use Universal Precautions. Do not use heat-inactivated, contaminated, or improperly stored specimens.

Note: See *SPECIMEN COLLECTION AND STORAGE* for important notes on proper methods to thaw frozen specimens. Proper sample handling is essential for accurate results.

Quidel suggests that normal plasma samples be diluted 1:10 in the provided Specimen Diluent; serum samples should be diluted 1:40. A 1:200 dilution, or greater, may be required for a sample with high levels of SC5b-9. Samples **must** be diluted so that A_{450} values observed are above the LLOQ and do not exceed the A_{450} value of the SC5b-9 Kit Plus Standard E. Samples with A_{450} readings outside this range should be re-assayed at a new dilution.

Determine the number (N) of specimens to be tested. Label test tubes #1 through #N, and record which specimen corresponds to each tube. Prepare an appropriate dilution (see preceding paragraph) of each specimen using the Specimen Diluent. Mix thoroughly, but avoid formation of foam and bubbles. Do not store or reuse 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 6 of *ASSAY PROCEDURE*). For small assay runs where only a few specimens are being tested, the diluted specimens and other reagents may be added directly to their assigned wells with a micropipette (100 μ L/well). For small or large runs, but especially larger runs, we recommend the use of a multichannel pipettor for adding specimens as follows. **(This procedure 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 μ L of each Standard, Control, or diluted specimen to the antibody-coated wells individually, 120 μ L to 130 μ L 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 μ L 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.

ASSAY PROCEDURE

Read entire product insert before beginning the assay.

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

1. Record the microassay well positions corresponding to the blank well(s), all test samples, Standards, and Controls as well as the indicated lot numbers from the vial labels. Label one corner of the Microassay Plate for orientation.
2. Prepare the microassay strips as follows:
 - a. Rehydrate microassay wells by adding approximately 300 μ L of Wash Solution to each well using a wash bottle or automated filling device.
 - b. Incubate at 15°C to 30°C for two minutes.
 - c. Remove the liquid from each well.
 - d. Invert the plate and tap firmly on absorbent paper twice to remove any remaining liquid.
3. Select one or more wells to serve as a blank. Add 100 μ L of Specimen Diluent to the well(s) that will be used to blank the plate reader.
4. Add 100 μ L of each SC5b-9 Standard (A, B, C, D, E) to duplicate wells. **Note: The standards have already been diluted and are ready to use.**

5. Add 100 μ L of both the SC5b-9 High Control and SC5b-9 Low Control to duplicate wells. **Note: The controls have already been diluted and are ready to use.**
6. Add 100 μ L of each diluted specimen to its assigned microassay well. (See *REAGENT PREPARATION*, Specimen Dilution).
7. Incubate at 15°C to 30°C for 60 \pm 1 minutes.
8. Wash the microassay wells as follows:
 - a. After the incubation in step 7 (or in step 10 below) remove the liquid from each well.
 - b. Add approximately 300 μ L Wash Solution to each well using a wash bottle or automated filling device.
 - c. Incubate the wells for 1 minute at 15°C to 30°C.
 - d. Remove the liquid from each well.
 - e. Add approximately 300 μ L Wash Solution to each well.
 - f. Remove the liquid 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 twice to remove any remaining liquid.
9. Using a multichannel or repeating pipette, dispense 50 μ L of SC5b-9 Plus Conjugate into each washed test well, including the blank well(s).
10. Incubate the microassay strips at 15°C to 30°C for 30 \pm 1 minutes.
11. Wash the microassay wells after the 30-minute incubation (step 10), as described under *ASSAY PROCEDURE*, step 8.
12. Immediately following the wash procedure, dispense 100 μ L of the Substrate Solution into each well, including the blank(s).
13. Incubate the microassay strips at 15°C to 30°C for 15 (\pm 1) minutes.
14. Add 100 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. Gently tap the plate to disperse the color development evenly. **NOTE: Optimal results may be obtained by using the plate reader's auto-mix function (if available) just prior to reading the plate.**
15. Determine the absorbance reading at 450 nm (A_{450} value) for each test well within 30 minutes after the addition of the Stop Solution (step 14), making the necessary blank correction.
16. Determine the concentration of samples and Controls from the standard curve.
17. Dispose of the remaining diluted specimens and controls and the used microassay strips (see *WARNINGS AND PRECAUTIONS*).

QUALITY CONTROL

Good laboratory practice recommends use of controls to ensure that the assay is performing properly. Each SC5b-9 Plus kit contains High and Low Controls that can be used for this purpose. 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. In addition, the package insert requires that the standard curve generated with the kit Standards meet stringent validation requirements. If the assay does not meet these requirements, repeat the assay, or contact Quidel Technical Service.

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 provided are to be used as a guideline only. The results obtained by your laboratory may differ.

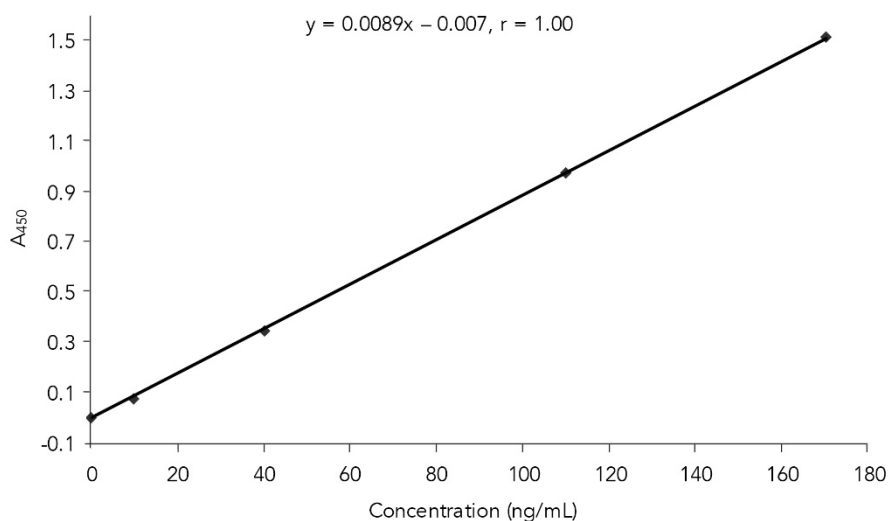
INTERPRETATION OF RESULTS

Calculation of Results

Use of the Standard Curve: The standard curve for the SC5b-9 Plus EIA is generated using the blank subtracted A_{450} values for each standard (on the y axis) and the assigned concentration for each standard (on the x axis). After linear regression, the generated standard curve must meet the validation requirements (see below). Most computers and calculators are capable of performing these calculations.

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

Figure 1
Representative Standard Curve



Sample	A_{450}	ng/mL
Standard A	0	0
Standard B	0.079	10
Standard C	0.347	40
Standard D	0.975	110
Standard E	1.512	170

Calculation of Actual SC5b-9 Concentration in Specimens

The assigned concentration on the standard vials and the control vials are absolute units of SC5b-9 complex. The concentration of SC5b-9 in a specimen is determined by multiplying the determined concentration by the appropriate specimen dilution factor. For example, if an EDTA-plasma specimen is diluted 1:10 for the assay and the linear regression curve yields a concentration of 20 ng SC5b-9/mL, then the concentration of SC5b-9 in the specimen would be 200 ng SC5b-9/mL (or 20 x 10).

In order to obtain accurate SC5b-9 concentration determinations for test specimens that yield A_{450} values greater than that of the SC5b-9 Standard E (or that yield A_{450} values less than the LLOQ), specimens should be re-assayed at a different dilution so that their new A_{450} values will be within these limits. In all repeat assays the SC5b-9 Standards and Controls must also be retested.

Validation

Determine the slope, intercept, and correlation coefficient of the derived best-fit line for the SC5b-9 A, B, C, D, and E Standards. The values must be within the specified ranges to qualify the assay:

correlation coefficient (r): > 0.95
slope (m): 0.0039 to 0.0123
y-intercept (b): (-)0.189 to (+)0.201

Refer to the vial labels for the acceptable SC5b-9 concentration range for the High and Low Controls.

LIMITATIONS OF THE PROCEDURE

The MicroVue SC5b-9 Plus Enzyme Immunoassay has been used to test specimens collected as serum or as plasma in EDTA and citrate. Other anticoagulants have not been tested.

SAMPLE VALUES

EDTA plasma and serum from forty (40) normal donors were tested in the SC5b-9 *Plus* EIA kit. The results are presented below:

	n	Mean (ng/mL)	Range: \pm 2SD (ng/mL)
EDTA Plasma	40	147	75 to 219
Serum	40	356	187 to 525

NOTE: the mean and Standard Deviation (SD) behavior of SC5b-9 complex concentrations determined for plasma or serum samples may vary between laboratories; therefore, it is recommended that each laboratory determines its own normal ranges. The values provided above should be regarded as a reference only.

PERFORMANCE CHARACTERISTICS

Limits

LOD: The limit of detection (LOD) for the SC5b-9 assay is 3.7 ng/mL, determined by the upper 3SD limit in a zero standard study.

LLOQ: The lower limit of quantitation (LLOQ) for the SC5b-9 assay is 8.8 ng/mL, the lowest concentration on the standard curve that met NCCLS criteria for accuracy and precision.

Interfering Substances

The following substances were tested in the SC5b-9 Plus assay and not found to interfere with the assay:

Substance	Concentration
Bilirubin	40 mg/dL
Hemoglobin	500 mg/dL
Triglycerides	3000 mg/dL
Li + Heparin	14 U/mL
Na + Heparin	14 U/mL
C9 Protein	180 mg/L
Albumin	6000 mg/dL
Glucose	1200 mg/dL
Cholesterol	500 mg/dL

Precision

Within-run and between-run precision was determined by assaying 20 replicates of 2 plasma samples and 2 serum samples in 10 different runs.

Sample	SC5b-9 (ng/mL)	Within-run ¹ C.V. (%)	Between-run ² C.V. (%)
Plasma	139.0	6.8	13.1
	462.9	1.9	5.2
Serum	803.6	2.8	10.4
	1410.6	1.6	5.0

¹n = 20 replicates

²n = 10 runs

Linearity

Linearity was performed by mixing a high plasma sample with a low plasma sample in various ratios to create intermediate levels of analyte. The average recovery was 94% with an absolute range of 86% to 104%.

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., 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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REF

A029 – MicroVue SC5b-9 Plus Kit

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PIA029001EN00 (10/16)

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



Biological risks

IVD

For *In Vitro* diagnostic use



Contains sufficient for 96 determinations

CONT

Contents/Contains

CONTROL

Control