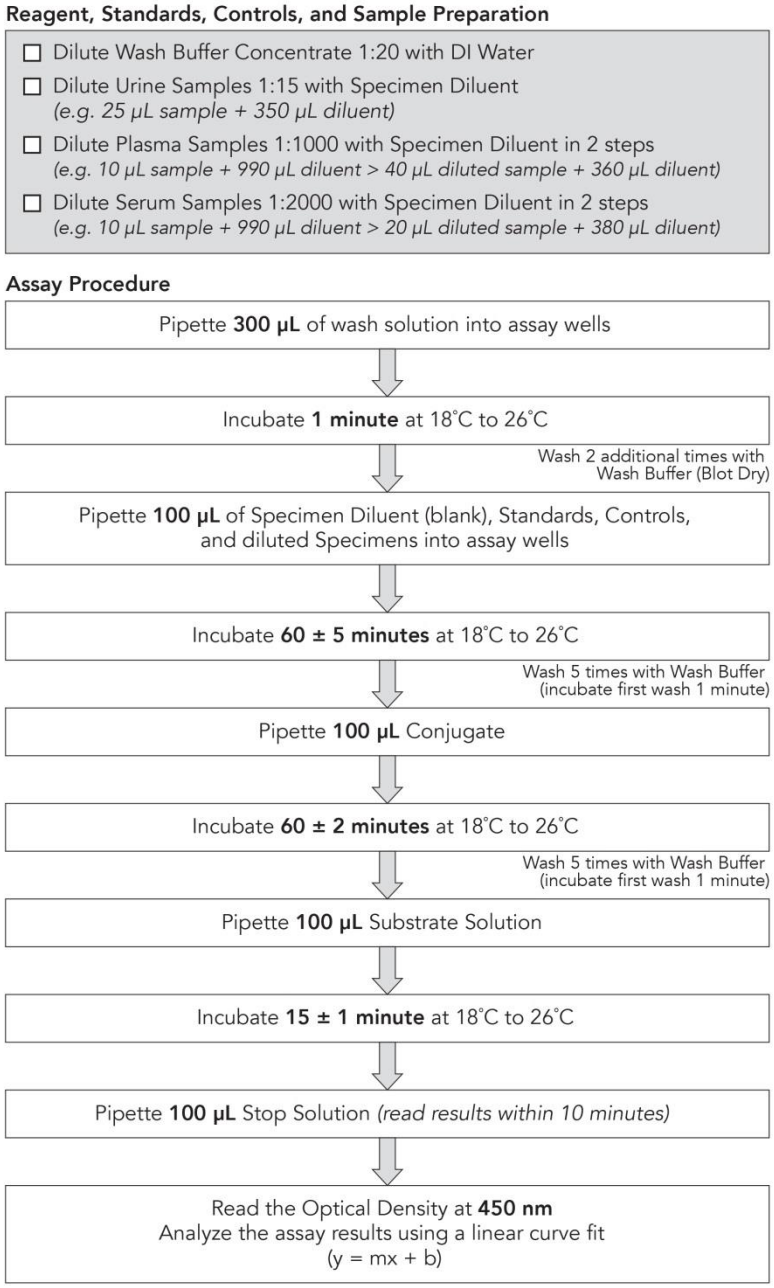
QUIDEL

MicroVue™ Complement Ba Fragment EIA

An immunoassay for the quantitation of the Ba fragment of Factor B, an indicator of the activation of the Alternative Complement Pathway, in human urine, plasma, and serum

For **Research Use Only** in the United States. Not for use in diagnostic procedures.

SUMMARY



SUMMARY AND EXPLANATION

The MicroVue Ba Enzyme Immunoassay Kit measures the amount of the complement fragment Ba, an activation fragment of Factor B of the alternative pathway of complement, in human urine, plasma or serum. Measurement of Ba in human urine, plasma or serum provides evidence of the involvement of the alternative pathway of complement.[1, 4, 14-22] The alternative complement pathway provides innate protection against microbial agents in the absence of specific antibody.[5-9] 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.

A centrally important reaction that occurs during alternative pathway activation is the conversion of the 93 Kd molecular weight Factor B zymogen to an active proteolytic enzyme. This is accomplished in a two-step reaction. During the first reaction step the Factor B forms a magnesium-dependent complex with C3(H20) or C3b.[8] The C3(H20),B complex is formed only in fluid-phase while the C3b,B complex can be formed either in fluid-phase or on a target surface.[5-8] Factor B, which is present in the C3(H20),B or the C3b,B complex, is cleaved into the Ba (33 Kd) and Bb (60 Kd) fragments in the second reaction step by the alternative pathway enzyme, Factor D.[5-8, 13]

Although alternative pathway activation is thought to occur primarily in the absence of specific antibody, many situations arise in which alternative pathway activation can occur as the result of classical pathway activation. For example, immune complexes that are present in autoimmune disease patients can trigger classical complement pathway activation with resultant production of C3b fragments. As described above, these C3b molecules are capable of binding Factor B and initiating its cleavage into the Ba and Bb fragments. Thus, alternative pathway activation can occur in antibody-mediated autoimmune disease states and may contribute significantly to enhanced complement activation and concomitant tissue destruction.

By assessing Factor B cleavage products in test specimens, one can estimate the extent of alternative pathway utilization occurring at the time of sample collection in the disease state under investigation. The MicroVue Ba EIA provides a simple, rapid, non-radioactive, highly specific, and quantitative procedure for measuring Factor B activation. It is ideal for investigations involving the role or status of the alternative complement pathway in numerous research and clinical settings and for monitoring the generation of Ba *in vitro*.

PRINCIPLE OF THE PROCEDURE

The MicroVue Ba Enzyme Immunoassay for the quantitation of Ba in human urine, plasma or serum is a three-step procedure utilizing (1) a microassay plate coated with a mouse monoclonal antibody that binds specifically to human Ba, (2) a HRP-conjugated polyclonal anti-human Factor B, and (3) a chromogenic substrate.

In Step 1, Standards, Controls, and test specimens are added to microassay wells pre-coated with a specific anti-Ba monoclonal antibody. Ba, but not Factor B or other complement activation products, present in the Standards, Controls or specimens will bind to the immobilized anti-Ba monoclonal antibody. After incubation, a wash cycle removes unbound material.

In Step 2, horseradish peroxidase (HRP)-conjugated polyclonal anti-Factor B antibody is added to each test well. The enzyme conjugated anti-Factor B binds to Ba captured in the microassay wells. After incubation, a wash cycle removes unbound, excess conjugate.

In Step 3, 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 Ba present in the test specimens, Standards, and Controls.

REAGENTS AND MATERIALS PROVIDED

96 Assays for the Ba fragment of Factor B
MicroVue Ba Enzyme Immunoassay kit contains the following:

A **Ba Standards:** **Parts 5159-5163** **1.5 mL each**
B Ready to use. Each contains human serum with assigned Ba concentration (ng/mL), protein stabilizers
C
D
E

L **Low Control** **Part 5164** **1.5 mL**
Ready to use. Contains human serum with assigned Ba concentration (ng/mL), protein stabilizers

H **High Control** **Part 5165** **1.5 mL**
Ready to use. Contains human serum with assigned Ba concentration (ng/mL), protein stabilizers

❶ **Microassay Plate** **Part 5166** **12 each**
Eight-well strips coated with a murine monoclonal antibody specific for human Ba in a resealable foil pouch

❷ **Stop Solution** **Part A9947** **12 mL**
Contains 1N (4%) Hydrochloric Acid

❸ **20X Wash Solution Concentrate** **Part A9957** **50 mL, 2 each**
Contains phosphate buffered saline (PBS), 1.0% Tween-20® and 0.035% Proclin® 300

❹ **Complement Specimen Diluent** **Part A3670** **50 mL**
Contains PBS, 0.05% Tween-20, 2.5% protein stabilizers, 0.035% ProClin 300

❺ **TMB Substrate** **Part 5059** **12 mL**
Ready to use. Contains 3,3',5,5'-tetramethylbenzidene (TMB) and Hydrogen Peroxide (H_2O_2)

❻ **Conjugate** **Part 5167** **12 mL**
Contains horseradish peroxidase-conjugated murine anti-human Ba 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)
- Clean, unused microassay plates, 96 well dilution plate and/or test tubes and racks
- Container for wash buffer dilution
- Wash bottle or other validated immunoassay washing system
- Micropipettes and pipette tips
- Adjustable multichannel pipette (8 or 12 channels) or repeating micropipettes
- Clean pipettes, 1 mL, 5 mL, and 10 mL
- Reagent reservoirs for adding conjugate, substrate and stop solutions to plate (use clean, unused reservoirs for each reagent)

- Plate reader capable of A_{450} readings between 0.0 and 3.0
- Deionized or distilled water

WARNINGS AND PRECAUTIONS

- For Research Use Only. Not for use in diagnostic procedures.
- 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 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."[23]
- 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 HANDLING AND PREPARATION*).
- Avoid microbial or cross-contamination of specimens or reagents.
- Test each sample in duplicate.
- Do not use any single microassay well for more than one test.
- 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 and contact with metal or rubber 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, 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*). 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.

INDICATIONS OF INSTABILITY OR DETERIORATION OF REAGENTS

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

SPECIMEN HANDLING AND PREPARATION

Handle and dispose of all specimens using Universal Precautions.

All specimen-handling operations should be carried out at 2°C to 8°C.

Specimen Collection

Serum/Plasma

Due to complement activation that occurs during clotting, the Ba concentration in normal human serum samples will be higher than the concentration obtained with EDTA plasma samples. The Ba levels in EDTA plasma may therefore more accurately represent *in vivo* concentrations.

The Ba fragment of Factor B is susceptible to proteolysis in improperly collected or stored specimens, and Ba may be generated in improperly handled specimens through artifactual complement activation; therefore, *the proper collection, storage, and handling of specimens is essential*.[24] For optimal plasma results, K2 EDTA collection tubes are recommended.

Serum and EDTA plasma specimens should be collected aseptically using standard techniques. The specimens should be tested immediately or stored on ice for no longer than two hours before being assayed.

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

Urine

The MicroVue Ba assay may be performed on preservative-free First Morning Void (FMV) or Second Morning Void (SMV) urine collections. Preferably, obtain collections prior to 10:00 am to obviate any potential influence of diurnal variation. Keep the urine sample refrigerated (2°C to 8°C) for storage of less than 1 day, or freeze the sample at ≤ –70°C for longer storage. Do not subject sample to more than 5 freeze/thaw cycles. **Avoid prolonged exposure to light, especially sunlight.** During routine processing, samples are not affected by normal, artificial laboratory lighting.

Thawing Frozen Specimens

To minimize specimen handling time, set up a dilution plate (or tubes), and add the appropriate volume of diluent (as described in the Specimen Dilution section below) prior to thawing specimens for evaluation.

Thaw frozen specimens rapidly at 37°C until just thawed. Transfer thawed specimens immediately to ice to prevent complement activation prior to dilution. **Keep specimens on ice for no longer than two hours. Do not leave specimens at 37°C, as complement activation may occur.** Do not thaw specimens at room temperature or on ice, as this can lead to complement activation and affect test outcome. Specimens should be tested as soon as possible after thawing. Up to five freeze thaw cycles may be performed without affecting the samples.

If samples need additional freezing for further analysis, Quidel suggests freezing multiple aliquots of the specimen to prevent exceeding the recommended number of freeze/thaw cycles.

Specimen Dilution

CAUTION: Treat all specimens as potentially infectious. Use Universal Precautions. Do not use heat – inactivated, contaminated or improperly stored specimens.

NOTE: See Thawing Frozen Specimens for important notes on proper methods to thaw frozen specimens. Proper sample handling is essential for accurate results.

Specimens must be diluted so that values observed are above the LLOQ and do not exceed the ULOQ. Specimens with readings outside this range should be re-assayed at a new dilution.

Prepare an appropriate dilution (see the following section) of each specimen using the Specimen Diluent. Mix each dilution gently to avoid formation of foam and bubbles. Do not store or reuse diluted specimens.

Urine

The recommended dilution for urine samples in Specimen Diluent is 1:15 for use in the MicroVue Ba Enzyme Immunoassay.

Plasma

The recommended dilution for plasma samples in Specimen Diluent is 1:1000. Perform the dilution in two steps (e.g., add 10 µL sample to 990 µL Specimen Diluent, then add 40 µL diluted sample to 360 µL Specimen Diluent).

Serum

The recommended dilution for serum samples in Specimen Diluent is 1:2000. Perform the dilution in two steps (e.g., add 10 µL sample to 990 µL Specimen Diluent, then add 20 µL diluted sample to 380 µL Specimen Diluent). Once diluted, the specimens must be added to the microassay wells within 30 minutes.

Specimens with high levels of complement activation may require larger sample dilutions than indicated above.

Adding Diluted Specimens to the Microtiter Wells

Complete the addition of diluted specimens to the microtiter wells within 5 minutes of the application of the first specimen. Either of two methods may be used to add diluted specimens, Standards, Controls, and buffer to the wells (see Step 4 of *ASSAY PROCEDURE*). For 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 µL/well). For small or large runs, but especially larger runs, Quidel recommends using the "replica plating" procedure described below to load the Standards, Controls, and diluted specimens into the microassay wells as rapidly as possible.

Use a multichannel pipettor to add 120-130 µL of each solution 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 being transferred.

This "replica plating" procedure may be used to conveniently add the Conjugate, Substrate, and Stop Solution, as well.

REAGENT PREPARATION

Bring all reagents and materials to 18°C to 26°C before use.
After removing the needed reagents and materials, return the unused items to their appropriate storage temperatures (see *STORAGE*).

Microassay Strips

Determine the number of strips needed for the assay. Quidel recommends testing the blank wells, Standards, and Controls in duplicate. Remove the unneeded strips, place them in the storage bag, reseal the bag, and return it to 2°C to 8°C. Secure the strips to be used in the assay in the assay plate frame.

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.

Ba Standards and Controls

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

ASSAY PROCEDURE

Read entire product Insert before beginning the assay.
See REAGENT PREPARATION and WARNINGS AND PRECAUTIONS before proceeding.

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 the wells for one minute at 18°C to 26°C.
   c. Aspirate the contents from each well.
   d. Add approximately 300 µL Wash Solution to each well.
   e. Aspirate the contents from each well.
   f. Repeat steps d-e one more time, for a total of three washes.
   g. Invert the plate and tap firmly on absorbent paper to remove any remaining liquid.
3. Select one or more wells to serve as a blank. Add 100 µL of Specimen Diluent to the well(s) that will be used to blank the plate reader.
4. Add 100 µL Standards, Controls, or diluted specimens to the assigned duplicate wells. **The entire plate must be loaded within 5 minutes of the first sample loaded onto the plate.**
5. Incubate at 18°C to 26°C for 60 ± 5 minutes.
6. Wash the microassay wells a total of 5 times using the following procedure:
   a. Aspirate 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. Incubate the wells for 1 minute at 18°C to 26°C.
d. Aspirate the contents from each well.
e. Invert the plate and tap firmly on absorbent paper to remove any remaining liquid.
f. Add approximately 300 µL Wash Solution to each well.
g. Aspirate the contents from each well.
h. Invert the plate and tap firmly on absorbent paper to remove any remaining liquid between each wash.
i. Repeat steps f-h three additional times for a total of five washes.
j. After the fifth wash cycle, invert the plate and tap firmly on absorbent paper to remove any remaining liquid.

7. Using a multichannel or repeating pipette, dispense 100 µL of Ba Conjugate into each washed test well, including the blank well(s).
8. Incubate the microassay strips at 18°C to 26°C for 60 ± 2 minutes.
9. Wash the microassay wells after the 60-minute incubation (step 8), as described under *ASSAY PROCEDURE*, step 6.
10. Immediately following the wash procedure, dispense 100 µL of the TMB Substrate Solution into each well, including the blank(s).
11. Incubate the microassay strips at 18°C to 26°C for 15 ± 1 minutes.
12. 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.
13. Gently tap the plate on the bench top to disperse the color development completely and evenly.
NOTE: Optimal results may be obtained by using the plate reader's auto-mix function (if available) just prior to reading the plate.
14. Determine the absorbance reading at 450 nm for each test well within 10 minutes after the addition of the Stop Solution (step 12), making a blank correction in accordance with the spectrophotometric system in use.
15. Determine the concentration of Samples and Controls from the standard curve.
16. Dispose of the remaining diluted specimens, Controls, substrate, and the used microassay strips (see *WARNINGS AND PRECAUTIONS*).

QUALITY CONTROL

The Certificate of Analysis included in this kit is lot specific and is to be used to verify that the results obtained by your laboratory are similar to those obtained at Quidel Corporation. The optical density values provided are intended as a guideline only. The results obtained by your laboratory may differ.

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

INTERPRETATION OF RESULTS

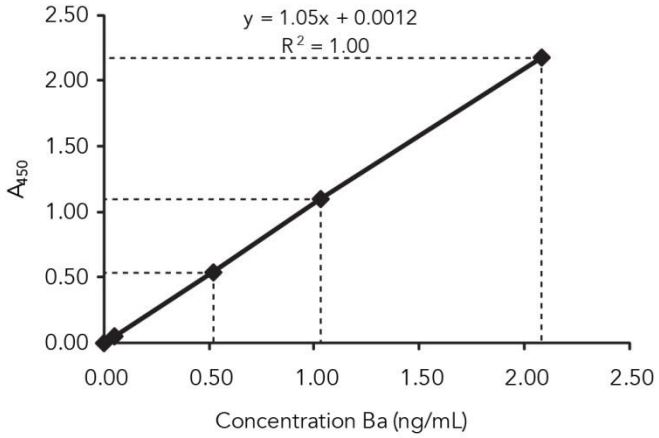
Use of the Standard Curve

The standard curve for the Ba EIA is generated using the blank subtracted A_{450} values for each Standard (on the y axis) and the assigned concentration for each Ba 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-fi t line of the standard curve. An example of a typical standard curve is shown in Figure 1.

Representative Standard Curve

Figure 1



Sample	A_{450}	ng/mL
Standard A	0.000	0
Standard B	0.052	0.05
Standard C	0.539	0.52
Standard D	1.098	1.03
Standard E	2.176	2.08
r = 1.000	m = 1.05	b = 0.0012

Calculation of Actual Ba Concentration in Test Specimens

The actual Ba concentration present in each undiluted test specimen is determined by multiplying the Ba ng/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 the values of the highest Standard (E), the results should be reported as “greater than” the Ba concentration of the highest Standard (E) multiplied by the specimen dilution factor. If a more accurate Ba concentration value is required, the test specimen should be re-assayed using a larger dilution factor. In all repeat assays, the Ba Standards and Controls must also be run.

VALIDATION

Determine the slope, intercept, and correlation coefficient of the derived best-fi t line. The values must be within the specified ranges to qualify the assay:

correlation coefficient (r):	≥ 0.98
slope (m):	0.52 to 1.63
y-intercept (b):	(-)0.05 to 0.05

Refer to the vial labels or product C of A for the mean acceptable Ba concentration ranges for the High and Low Controls.

LIMITATIONS OF THE PROCEDURE

The MicroVue Ba Enzyme Immunoassay has been used to test specimens collected as urine, serum, or as plasma in K2 EDTA. Other anticoagulants have not been tested.

OBSERVED VALUES

EDTA plasma, serum, and urine from 35, 29, and 16 normal donors, respectively, were tested in the MicroVue Ba Enzyme Immunoassay kit. The results are presented below.

	n	Mean	Range (ng/mL)
EDTA Plasma	35	658	226 to 2153
Serum	29	1642	436 to 3362
Urine	16	7.7	0.6 to 27.0

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

PERFORMANCE CHARACTERISTICS

Limits

LOD: The limit of detection (LOD) for the Ba EIA is 0.011 ng/mL, determined by the upper 3SD limit in a zero standard study.

LLOQ: The lower limit of quantitation (LLOQ) for the Ba EIA is 0.033 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 Ba EIA is 3.239 ng/mL, the highest concentration that met CLSI criteria for accuracy and precision.

Interfering Substances

The following substances were tested in the Ba EIA and found to not interfere with the assay using plasma or serum samples:

Substance	Concentration
Bilirubin	40 mg/dL
Hemoglobin	500 mg/dL
Triglycerides	3000 mg/dL
Glucose	1200 mg/dL
Cholesterol	500 mg/dL
Albumin	6000 mg/dL
Gamma Globulin	6000 mg/dL

The following substances were tested in the Ba EIA and found to not interfere with the assay using urine samples:

Substance	Concentration
Acetone	1000 mg/dL
Creatinine	500 mg/dL

Glucose	2000 mg/dL
Bilirubin	0.25 mg/dL
Hemoglobin	200 mg/dL
Urea	6000 mg/dL

The following substances *were* found to interfere with the assay using urine samples:

Substance	Concentration
Albumin	340 mg/dL
Sodium Chloride	170 mg/dL

Precision

Intra- and inter-assay precision was determined by assaying 20 replicates of 1 plasma sample, 1 serum sample and 2 urine samples in 10 different assays.

Sample	Ba (ng/mL)	Intra-assay[1] C.V. (%)	Inter-assay[2] C.V. (%)
EDTA Plasma	376.0	3.3	2.4
Serum	1111	2.3	8.1
Urine	1.925	2.2	7.6
	22.98	2.2	3.4

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

Linearity

Linearity was performed by diluting samples with specimen diluent and comparing observed values with expected values. Typical results are provided below.

Sample	Dilution Factor	Observed Ba (ng/mL)[3]	Expected Ba (ng/mL)[3]	Recovery (%)
EDTA Plasma	1:1000	0.634	0.634	100.0
	1:1500	0.424	0.423	100.3
	1:2000	0.323	0.317	101.9
	1:3000	0.219	0.211	103.6
Serum	1:2000	1.464	1.464	100.0
	1:3000	0.986	0.976	101.0
	1:4000	0.734	0.732	100.3
	1:5000	0.580	0.586	99.0
Urine	1:15	0.106	0.106	100.0
	1:20	0.078	0.0795	98.1
	1:25	0.064	0.0636	100.6
	1:30	0.058	0.053	109.4

[3]Dilution factor not included

Spike Recovery

Spike Recovery was performed by spiking samples with a known quantity of purified Ba and comparing observed values with expected values.

Sample	Ba (ng/mL)	Spike (ng/mL)	Result (ng/mL)	Recovery (%)

Plasma 1	593.1		857.8	103.8
Plasma 2	356.6	233.3	646.4	109.6
Plasma 3	590.0		845.5	102.7
Serum 1	1194		1664	99.0
Serum 2	2088	486.4	2648	102.8
Serum 3	2497		2953	99.0
Urine 1	1.115		4.026	92.5
Urine 2	1.711	3.237	4.891	98.8
Urine 3	21.37		23.97	97.4

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

1. Wan K.C, Lewis W.H.P, Leung P.C., Chien P. Hung L.K. 1998. "A longitudinal study of C3, C3d and factor Ba in burn patients in Hong Kong." *BURNS* 24(3): 241-44.
2. Sundsmo J., Chin J., Papin R., Fair D., Werb Z. 1985. "Factor B, The complement alternative pathway serine proteinase, is a major constitutive protein synthesized and secreted by resident and elicited mouse macrophages." *J. EXP. MED* 161:306-22.
3. Ueda A., Kearney J., Roux K., Volanakis J. 1986. "Probing functional sites on complement protein B with monoclonal antibodies." *J. IMMUNOLOGY* 138 (4):1143-49.
4. Ambrus J., Peters M., Fauci A., Brown E. 1990. "The Ba fragment of complement factor B inhibits human B lymphocyte proliferation." *J. IMMUNOLOGY* 144(5):1549-53.
5. Schreiber, R.D. and Muller-Eberhard, H.J. 1980. New developments in the activation of the alternative pathway of complement. in *IMMUNOASSAYS: CLINICAL LABORATORY TECHNIQUES FOR THE 1980'S*. Alan R. Liss, Inc., New York. 411-31.
6. Gotze, 0. and Muller-Eberhard, H.J. 1976. The alternative pathway of complement activation. *ADV. IMMUNOL.* 24:1-35.
7. Fearon, D.T. and Austen, K.F. 1980. Current concepts in immunology: the alternative pathway of complement – a system for host resistance to microbial infection. *NEW ENGL. J. MED.* 303(5):259-63.
8. Pangburn, M.K. and Muller-Eberhard, H.J. 1984. The alternative pathway of complement. *SPRINGER SEMIN IMMUNOPATHOL* 7:163.
9. Ratnoff , W.E., Fearon, D.T., and Austen, K.F. 1983. The role of antibody in the activation of the alternative complement pathway. *SPRINGER SEMIN IMMUNOPATHOL* 6:361.
10. Hugli, T.E. 1986. Biochemistry and biology of anaphylatoxins. *COMPLEMENT* 3:111-27.
11. Fearon, D.T. and Austen, K.F. 1977. Activation of the alternative complement pathway due to resistance of zymosanbound amplifi cation convertase to endogenous regulatory mechanisms. *PROC. NATL. ACAD. SCI.* (USA). 74(4):1683-87.
12. Fearon, D.T. 1979. Regulation of the amplification C3 convertase of human complement by an inhibitory protein isolated from human erythrocyte membrane. *PROC. NATL. ACAD. SCI.* (USA). 76(11): 5867-71.
13. Fishelson, Z. and Muller-Eberhard, H.J. 1984. Residual hemolytic and proteolytic activity expressed by Bb after decay-dissociation of C3b, Bb. *J. IMMUNOL.* 132(3):1425-29.

14. Gotze, O., Bianco, C. and Cohn, Z.A. 1979. The induction of macrophage spreading by Factor B of the properdin system. *J. EXE. MED.* 149:372.
15. Kolb,W.P., Morrow, P.R. and Tamerius, J.D. 1989. Ba and Bb Fragments of Factor B activation: Fragment production, biological activities, neoepitope expression and quantitation in clinical samples. *COMPLEMENT AND INFLAMMATION* 6:175.
16. Sefton, M.V., et al. 1994. "Using ELISA to evaluate complement activation by reference biomaterials." *J. MAT. SCI* 5:622-27.
17. Mold, C. Tamerius, J.D., et al. 1995 "Complement activation during painful crisis in sickle cell anemia" *CLINICAL IMMUNOLOGY AND IMMUNOPATHOLOGY* 70(3):314-20.
18. Manzi, S. Rairie, J. et al. 1996 "Sensitivity and Specificity of plasma and urine complement split products as indicators of lupus disease activity" *ARTHRITIS AND RHEUMATISM* 39(7):1178-88.
19. Buyon J, Tamerius J. et al. 1992. "Assessment of Disease activity and impending fl are in patients with systemic lupus erythematosis" *ARTHRITIS AND RHEUMATISM* 35(9):1028-36.
20. Oppermann M., Kurts C., Zierz R., Quentin E., Weber M., and Gotze O. 1991. "Elevated plasma levels of the immunosuppressive complement fragment Ba in renal failure." *INTL. SOC NEPHROLOGY* 40:939-47.
21. Pryzdial E., Isenman D. 1987. "Alternative complement pathway activation fragement Ba binds to C3b." *JBC* 262(4):1519-25.
22. Buyon J., Tamerius J., Ordorcia S., Young B., Abramson S. 1992. "Activation of the alternative complement pathway accompanies disease fl ares in systemic lupus erythematosus during pregnancy." *ARTH. AND RHEUM.* 35(1):56-61.
23. U.S. Department of Health and Human Services Centers for Disease Control and Prevention and National Institutes of Health. 2007. *BIOSAFETY IN MICROBIOLOGICAL AND BIOMEDICAL LABORATORIES (BMBL) 5TH EDITION.* Washington: U.S. Government Printing Offi ce. http://www.cdc.gov/od/ohs/biosfty/bmbl5/bmbl5toc.htm.
24. Mollnes, T.E., P. Garred, and G. Bergseth. 1988. Effect of time, temperature and anticoagulants on *in vitro* complement activation: Consequences for collection and preservation of samples to be examined for complement activation. *CLIN. EXP. IMMUNOLOGY*. 73:484-88.

REF A033 – MicroVue Ba Fragment EIA Kit

RUO

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PIA033000EN00 (11/16)

GLOSSARY

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