



Multiplex

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

Background

The Complement System consists of more than 50 fluid and membrane-bound proteins and protein fragments organized into four primary pathways: the Classical, Alternative, Lectin and Terminal. Through specific activation steps complement proteins mediate a set of activities including inflammation, clearance of immune complexes, disruption of cell membranes, and regulation of the immune response. Deficiencies in the complement cascade can predispose individuals to infection through ineffective opsonization or defects in the ability to lyse invading pathogens. Also, the complement system is associated with many acute and chronic diseases due to abnormalities in its function. Quantitative analysis of different complement proteins and their activation products are of great importance in experimental and clinical medicine. The ability to measure multiple complement proteins in an individual sample constitutes a major step forward in research-based tools. The MicroVue Complement Multiplex can quantitatively measure eight individual complement proteins, providing a comprehensive overview of complement system homeostasis and activation.

Format

- 96-well microplate
- Standards and Controls (High and Low)
- **Samples:** Plasma / Serum
- **Sample Volume:** 50 µL (1:100 dilution for plasma or 1:1000 for serum – consult manual)
- **Assay Time:** 3.5 Hours

Assay Steps

- Sample volume per well (50 µL)
- Sample Dilution (1:100)
- Antigen incubation time (2 hours)
- Wash wells (3X)
- Detection volume per well (50 µL)
- Detection incubation time (1 hour)
- Wash wells (3X)
- SHRP 1X volume per well (50 µL)
- SHRP 1X incubation time (20 minutes)
- Wash wells (6X)
- Substrate volume per well (50 µL)
- Imaging Time (300 sec on Q-View Imager PRO or 270 sec on Q-View Imager LS)

Assay Performance (plasma samples only)

Analyte	Ba (ng/mL)	Bb (µg/mL)	C3a (ng/mL)	C4a (ng/mL)	C4d (µg/mL)	C5a (ng/mL)	Factor H (µg/mL)	Factor I (ng/mL)	SC5b-9 (ng/mL)
Limit of Detection (LOD)	0.113	0.0018	0.0103	0.145	0.0034	0.002	0.063	17.16	0.95
Lower Limit of Quantitation	0.38	0.003	0.55	0.8	0.0154	0.0082	0.17	39.40	1.63
Upper Limit of Quantitation	28.00	0.24	87.80	87.00	0.90	1.10	8.60	748.10	362.10
Precision (intra-assay)	6%	10%	5%	7%	4%	6%	3%	5%	6%
Precision (inter-assay)	11%	13%	10%	10%	6%	10%	9%	10%	10%
Correlation to MicroVue Kit (R ²)	0.928	0.977	0.966	0.969	0.920	0.962	0.978	0.884	0.962

Performance and Validation Data (plasma samples only)

The MicroVue Complement EIA kits are first-in-class products for complement research. The approach in developing the MicroVue Complement Multiplex was that it would perform in a comparable manner to our existing EIA products.

The table below shows how the various specifications compare between the Complement Multiplex and the individual analyte kits. Because the individual kits differ on dilution factors and other specifications, an “adjusted” set of specifications is provided for direct comparison between the Multiplex assay and the individual analyte kits.

Analyte	Units	Format*	Dilution Factor	Limit of Detection (LOD)	Lower Limit of Quantitation (LLOQ)	Upper Limit of Quantitation (ULOQ)	Precision (intra-assay)	Precision (inter-assay)
Ba	ng/mL	Multiplex	1:100	0.1129	0.38	28	6%	11%
		MicroVue – Adjusted	1:100	0.11	0.33	32.39		
		MicroVue – Kit Specs	1:1000	0.011	0.033	3.239	2.2% – 2.3%	2.4% – 8.1%
Bb	µg/mL	Multiplex	1:100	0.0018	0.003	0.24	10%	13%
		MicroVue – Adjusted	1:100	0.0018	0.0033	0.0836	2.4% – 4.0%	6.2% – 9.1%
		MicroVue – Kit Specs	1:10	0.018	0.033	0.836		
C3a	ng/mL	Multiplex	1:100	0.0103	0.55	87.8	5%	10%
		MicroVue – Adjusted	1:100	0.024	0.046	5.062	4.5% – 5.3%	5.9% – 19.6%
		MicroVue – Kit Specs	1:200	0.012	0.023	2.531		
C4a	ng/mL	Multiplex	1:100	0.1496	0.8	87	7%	10%
		MicroVue – Adjusted	1:100	0.116	2.0	24.4	3.7% – 4.3%	4.0% – 4.4%
		MicroVue – Kit Specs	1:40	0.29	5.0	61		
C4d	µg/mL	Multiplex	1:100	0.0034	0.0154	0.90	4%	6%
		MicroVue – Adjusted	1:100	0.028	0.0154	N/A	6.1% – 9.7%	8.5% – 11.2%
		MicroVue – Kit Specs	1:70	0.001	0.022	N/A		
C5a	ng/mL	Multiplex	1:100	0.002	0.0082	1.1	6%	10%
		MicroVue – Adjusted	1:100	0.002	0.01	N/A	3.5% – 3.9%	7.1% – 13.0%
		MicroVue – Kit Specs	1:20	0.01	0.05	N/A		
Factor H	µg/mL	Multiplex	1:100	0.063	0.17	8.6	3%	9%
		MicroVue – Adjusted	1:100	0.15775	0.232	26.05	4.1% – 5.2%	9.0% – 9.7%
	ng/mL	MicroVue – Kit Specs	1:5000	3.155	4.64	521		
Factor I	ng/mL	Multiplex	1:100	17.1565	39.4	748.1	5%	10%
		MicroVue – Adjusted	1:100	7.5	24	1231.5	3%	5%
		MicroVue – Kit Specs	1:1500	0.5	1.6	82.1		
SC5b-9	ng/mL	Multiplex	1:100	0.9435	1.63	362.1	6%	10%
		MicroVue – Adjusted	1:100	0.37	0.88	N/A	1.6% – 6.8%	5.0% – 13.1%
		MicroVue – Kit Specs	1:10	3.7	8.8	N/A		

*The table shows three analyte formats:

Multiplex The specifications from the Complement Multiplex assay.

MicroVue – Adjusted The specifications from the individual MicroVue analyte kit, adjusted for direct comparison to the Multiplex assay. The adjusted specifications are Dilution Factor, LOD, LLOQ, and ULOQ.

MicroVue – Kit Specs The specifications from the individual MicroVue analyte kit.