



Multiplex – Panel 1

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 twelve individual complement proteins, providing a comprehensive overview of complement system homeostasis and activation.

Analytes

Panel 1 analytes include Ba, Bb, C2 Intact, C3a, C3d, C4a, C4d, C5a, sC5b-9, Factor D, Factor H, and Factor I.

Compatibility

For Panel 1 custom kits certain analytes are not compatible and cannot be ordered together in the same assay:

- C3a and C3d are not compatible
- C4a and C4d are not compatible

Format

- 96-well microplate
- Standards and Controls (High and Low)
- **Samples:** Serum / Plasma
- **Sample Volume:** 50 µL (consult the Package Insert for dilution instructions for all sample types)
- **Assay Time:** 3.5 Hours

Assay Steps

- Sample volume per well (50 µL)
- Sample Dilution
- 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 (250-300 sec)

Assay Performance

Analyte	Units	Limit of Detection (LOD)	Lower Limit of Quantitation (LLOQ)	Upper Limit of Quantitation (ULOQ)	Precision (intra-assay)	Precision (inter-assay)	Correlation (R ²)*
Ba	ng/mL	0.14	0.34	29.2	8%	12%	0.94
Bb	µg/mL	0.0022	0.0030	0.20	10%	12%	0.92
C2 Intact	ng/mL	0.62	1.24	586.2	9%	12%	0.97
C3a	ng/mL	0.17	0.36	85.0	7%	13%	0.93
C3d	ng/mL	1.18	1.10	479.4	12%	10%	0.98
C4a	ng/mL	0.17	0.68	44.6	4%	8%	0.86
C4d	µg/mL	0.0030	0.0060	3.4	9%	15%	0.90
C5a	ng/mL	0.0025	0.017	1.85	10%	13%	0.96
sC5b-9	ng/mL	1.13	1.86	617.0	10%	14%	0.98
Factor D	ng/mL	0.014	0.070	39.9	12%	12%	0.97
Factor H	µg/mL	0.25	0.29	6.1	9%	12%	0.92
Factor I	ng/mL	20.15	16.67	497.1	12%	15%	0.91

*Analytes Ba, Bb, C3a, C4a, C4d, C5a, sC5b-9, Factor H, and Factor I were correlated to their corresponding MicroVue single analyte kits. Analytes C2 Intact C3d, and Factor D were correlated to a series of AAA characterized proteins that were tested in duplicate. The 19 plasma samples and AAA characterized proteins were tested on at least three different days.