



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

The MicroVue C4a EIA provides quantitative measurement of the complement fragment C4a, an activation fragment of complement protein C4, in experimental samples.

Under normal conditions, activation of the classical or lectin complement pathways results in the cleavage of the complement protein, C4 into C4a and C4b by the protease C1s. C4a is rapidly cleaved to its more stable, less active form C4a-des Arg by endogenous serum carboxypeptidase N enzyme. Thus, quantitation of C4a-des Arg provides a reliable measurement of classical or lectin complement pathway activation that has occurred in test samples.

Format

- ELISA
- 96-well microplate with reagents sufficient to test 40 samples in duplicate
- Sample type: Serum, K2 or K3 EDTA plasma or other experimental samples
- Controls: High, low included

Species Cross Reactivity:

- Rhesus Macaque, Cynomolgus Monkey, African Green Monkey, Human
- No reactivity with Cow, Chicken, Goat, Mouse, Rabbit, Rat, Sheep, Guinea Pig

Specimen

- Samples collected to avoid hemolysis
- Heparin or citrated plasma samples are not recommended

Assay Steps

- Prepare Reagents, Standards, Controls and Samples
- Add 300 µL of wash solution into assay wells
- Incubate 1 minute
- Wash the assay wells three times
- Add 100 µL of all Standards, Controls and diluted samples into assay wells
- Incubate 60 minutes at 15°C to 27°C
- Wash the assay wells 4 times
- Add 100 µL of C4a Conjugate to each well
- Incubate 60 minutes at 15°C to 27°C
- Wash the assay wells 4 times
- Pipette 100 µL Substrate Solution
- Incubate 15 minutes at 15°C to 27°C
- Add 100 µL of Stop Solution to each assay well
- Measure absorbance at 450 nm

Assay Performance

Method: ELISA

Specimen Volume:

Plasma: Maximum of 10 µL

Serum: Minimum of 10 µL

Analyte: C4a

Limit of Detection: 0.29 ng/mL

Assay range: 5.0-61 ng/mL

Precision (inter-assay): 4.0%-4.4%

Precision (intra-assay): 3.7%-4.3%

Assay Time: Approx. 3 hours

Specificity: C4a 100%

C4 0.47%

C3 0.13%

C5 0.54%

C3a 0.64%

C5a 2.86%