



### C4d Fragment

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

#### Background

The MicroVue C4d Enzyme Immunoassay provides quantitative measurement of C4d containing fragments.

C4d is generated as a result of cleavage of complement protein C4 upon activation of the classical complement pathway. Activation of the classical pathway is triggered by antigen-antibody complexes or other substances such as retroviruses, bacteria and parasites binding to the C1q component of C1. Subsequently, the zymogen C1s subunit is activated and converted into an active proteolytic enzyme which cleaves the C4  $\alpha$ -chain resulting in the production of C4a and C4b fragments. C4b is rapidly cleaved by Factor I yielding inactive C4b or C4bi. Further degradation yields the fragments C4c and C4d.

The levels of C4d, normalized by levels of native C4, can be significantly elevated in samples from patients with rheumatoid arthritis, hereditary angioedema (HAE), systemic lupus erythematosus (SLE), and may also be elevated in patients with a variety of other autoimmune diseases in which activation of the classical complement pathway is known to occur.

#### Format

- 96-well microplate with reagents sufficient to test 40 samples in duplicate
- Standards and Controls

#### Assay Steps

- Dilute Wash Buffer and reconstitute Standard and Controls
- Dilute Samples 1:70 with Complement Specimen Diluent
- Pipette ~300  $\mu$ L of wash solution into assay wells
- Incubate 1 minute at 15°C to 30°C
- Wash 2 times with Wash Buffer
- Pipette 100  $\mu$ L of Complement Specimen Diluent, Standards, Controls and Samples into assay wells
- Incubate 30  $\pm$ 1 minute at 15°C to 30°C

- Wash assay wells 5 times with Wash Buffer
- Pipette 50  $\mu$ L C4d Conjugate
- Incubate 30  $\pm$ 1 minute at 15°C to 30°C
- Prepare 1X Substrate Solution
- Wash assay wells 5 times with Wash Buffer
- Pipette 100  $\mu$ L 1X Substrate Solution
- Incubate 30  $\pm$ 1 minute at 15°C to 30°C.
- Add 50  $\mu$ L of Stop Solution to each assay well
- Read the Optical Density at 405 nm

#### Assay Performance

**Method:** Direct Capture

**Samples:** Diluted 1:70

**Sample Volume:** 100  $\mu$ L

**Precision (intra-assay):**  
6.1% to 9.7%

**Precision (inter-assay):**  
8.5% to 11.2%

**Assay Time:** < 2 hours

**LOD:** 0.001  $\mu$ g/mL

**LLOQ:** 0.022  $\mu$ g/mL

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MicroVue C4d Fragment EIA – Cat. #A008 (CE)  
Cat. #A009 (RUO)

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Also available:

MicroVue iC3b EIA – Cat. #A006 (RUO)

MicroVue C3a Plus EIA – Cat. #A031 (RUO)

Cat. #A032 (CE)

MicroVue SC5b-9 Plus EIA – Cat. #A020 (RUO)

Cat. #A029 (CE)

Monoclonal Antibody to Human C4d – Cat. #A239

