

## Monoclonal Antibodies: Anti-Human C4/C4d

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

### Background

Activation of the classical complement pathway begins with the binding of an activating substance (e.g. immune complex) to the C1q molecule of C1. This, in turn, activates the C1r(2)C1s(2) sub-units, resulting in cleavage of C4 to C4b near the amino terminus of the gamma chain releasing C4a in the process. The short-lived C4b molecule can bind covalently to membranes or other surfaces via either an amide or ester bond. This is an inefficient process that is limited to the immediate vicinity of the C1 complex. C4b then takes part in the classical convertase enzyme. Because of the short life of the C4b molecule much of the C4d is free and circulates in serum.

Both bound and free C4b are strictly controlled *in vivo*. The ability of C4b to participate in classical pathway activation and opsonization reactions is inhibited by a single site cleavage of the alpha chain by Factor I. This reaction requires either C4 binding protein or CR1 as a cofactor. This initial cleavage inactivates C4b resulting in iC4b. Further degradation of this molecule by Factor I produces the C4c and C4d fragments. Both of these fragments can be produced in fluid phase or on target surface.

### Specificity

The specificity of the monoclonal antibody was established via a series of immunoassays. The antibody was shown by ELISA to bind highly purified C4d and intact C4, but not to purified C4c. Additionally, the antibody was shown to immunoprecipitate the C4d fragment using radiolabeled C4d with protein A-bearing bacteria, but not the C4c fragment.

### Applications

Because specific techniques differ from lab to lab, the provided information should be used as a guideline only. As C4b has a short half life *in vivo*, C4d is an excellent marker for classical complement activation *in vivo* or *in vitro* and is therefore the basis of the MicroVue C4d EIA Kit (Cat. #A008).

Applications of this antibody have been evaluated by various research facilities, and include EIA,<sup>1</sup> Western Blot,<sup>2</sup> IHC,<sup>2,4</sup> and FACS.<sup>5,6</sup>

### Specifications

- Volume/vial: 100 µL or 0.5 mL
- Storage: 2°C to 8°C\* (≤ 30 days)
- Concentration: 1.0-1.2 mg/mL
- Buffer: Borate Buffered Saline
- Isotype: IgG<sub>1</sub>bk

### Species Cross Reactivity:

- Human, porcine.<sup>7</sup> No cross-reactivity was observed with specimens from baboon, bovine, cat, chicken, dog, guinea pig, hamster, horse, mouse, rabbit, rat, or sheep.

\*For long-term storage (> 30 days), aliquot and store at ≤ -20°C. Avoid repeated freeze-thaw.

### References

- <sup>1</sup>Barbosa, A., et al. Immune evasion of *Lptospira* species by acquisition of human complement regulator C4BP. *Infection and Immunity* 77.3:1137-143 (2009)
- <sup>2</sup>Yasojima, K., et al. Up Regulated Production and Activation of the Complement System in Alzheimer's Disease Brain. *Am J Path* 154:927-935 (1999).
- <sup>3</sup>Stoltzner, S.E. Temporal Accrual of Complement Proteins in Amyloid Plaques in Down's Syndrome with Alzheimer's Disease. *Am J Path* 156:489-499 (2000).
- <sup>4</sup>Desvaux, D., et al. Acute Renal Allograft Rejection with Major Interstitial Oedema and Plasma Cellrich Infiltrates: High Á-interferon Expression and Poor Clinical Outcome.

- Nephrol Dial Transplant 19:933-939 (2004).
- <sup>5</sup>Nordstrom, T., et al. The Emerging Pathogen *Moraxella Catarrhalis* Interacts with Complement Inhibitor C4b Binding Protein through ubiquitous surface proteins A1 and A2. *J Immunol* 173:4598-4606 (2004).
- <sup>6</sup>Gemmell, C. A Flow Cytometric Immunoassay to Quantify Adsorption of Complement Activation Products on Artificial Surfaces. *J Biomed Mater Res* 37, 474-480 (1997).
- <sup>7</sup>Pascher, A., et al., Immunopathological observations after xenogenic liver perfusions using donor pigs transgenic for human decay-accelerating factor 1,2. *Transplantation* 64.3: 384-91 (1997)

Also available:

MicroVue C4d EIA Kit – Cat. #A008

Biotinylated Monoclonal Anti-Human C4(C4d) – Cat. #A704

Monoclonal Anti-Human C4d (neoantigen) – Cat. #251

Monoclonal Anti-Human C4(C4c) – Cat. #A211

Purified Human C4 Protein – Cat. #A402