Monoclonal Mouse Anti-Human C5b-9
Clone aE11
Code No. M 0777

For research use only. Not for use in diagnostic procedures.

Recommended use
Monoclonal Mouse Anti-Human C5b-9, Clone aE11, is recommended for use in immunocytochemistry. The antibody labels the Poly (C9) component in the C5b-9 complex in various tissues and is a useful tool for the identification of complement activation.

Synonym for antigen
Terminal complement complex, TCC (1); Membrane attack complex, MAC (2).

Introduction
Activation of the complement system plays a key role in normal inflammatory response to injury but may cause substantial injury when activated inappropriately. The complement system is activated either through the classical (antibody induced) or the alternative (microbial surface, polysaccharide induced) pathway, both leading to the formation of the C5b-9 complex. Fluid-phase binding of the multifunctional glycoprotein S-protein (vitronectin) to C5b-9 leads to the formation of a cytolytically inactive complex, SC5b-9, which is unable to attach to cells (3, 4). For review of the complement system (2, 3).

Reagent provided
Monoclonal mouse antibody provided in liquid form as cell culture supernatant dialysed against 0.05 mol/L Tris/HCl, pH 7.2, and containing 15 mmol/L NaN₃.

Immunogen
Purified human MAC (1).

Specificity
The clone was initially selected in an ELISA demonstrating reactivity towards the immunizing agent and activated serum (1).

In Western blotting of purified components of the TCC the antibody labels the poly-C9 and to a lesser degree the C9 but do not recognize the C5, C6, C7 or the C8 components (1).
The antibody reacts with a neoepitope on poly C9 complement factor. This neoepitope is exposed in the solid-phase and membrane form and in the fluid-phase form of the terminal complement complex, but not in native C9.
The antibody reacts with both membrane-bound and adsorbed terminal complement complex (5).
As demonstrated in immunohistochemistry and ELISA, the antibody cross-reacts with the C5b-9 equivalent protein in various mammals, including baboon (6), rat (7), swine (8).

Precautions
1. The device is not intended for clinical use including diagnosis, prognosis, and monitoring of a disease state, and it must not be used in conjunction with patient records or treatment.
2. This product contains sodium azide (NaN₃), a chemical highly toxic in pure form. At product concentrations, though not classified as hazardous, sodium azide may react with lead and copper plumbing to form highly explosive build-ups of metal azides. Upon disposal, flush with large volumes of water to prevent metal azide build-up in plumbing.
3. As with any product derived from biological sources, proper handling procedures should be used.

Storage
Store at 2-8 °C. Do not use after expiration date stamped on vial. If unexpected staining is observed which cannot be explained by variations in laboratory procedures and a problem with the antibody is suspected, contact our Technical Services.

Specimen preparation
Paraffin sections: The antibody cannot be used for labelling formalin-fixed, paraffin-embedded tissue sections.
Frozen sections and cell preparation: The antibody can be used for labelling frozen sections.

Staining procedure
Dilution: Monoclonal Mouse Anti-Human C5b-9, code No. 0777, may be used at a dilution range of 1:25-1:50 when applied on acetone-fixed cryostat sections of tonsil or kidney and using 30 minutes incubation at room temperature with the primary antibody. Optimal conditions may vary depending on specimen and preparation method, and should be determined by each individual laboratory. The recommended negative control is Dako Mouse IgG2a, code No. X 0943, diluted to the same mouse IgG2a concentration as the primary antibody.
Visualization: DAKO LSAB™+/HRP kit, code No. K 0679, and DAKO EnVision™+/HRP kits, code Nos. K 4004 and K 4006, are recommended. For frozen sections and cell preparations, Dako APAAP kit, code No. K 0670, is a good alternative if endogenous peroxidase staining is a concern. Follow the procedure enclosed with the selected visualization kit.

Performance characteristics
Normal tissues: The antibody demonstrates positive staining for the C9 neoepitope of TCC on follicular dendritic cells in germinal centres of secondary lymphoid follicles of reactive lymphoid tissues (9). Tubuli of normal kidney show a scattered weak staining, while glomeruli are negative (1). This antibody also detects
SC5b-9 produced by alveolar macrophages in serum-free cultures (10). It reveals strong granular staining of deposits of TCC in kidney biopsies (e.g. glomeruli and tubuli).

References
2. Makrides