Monoclonal Mouse Anti-Human C5b-9
Clone aE11

Code M0777

Intended use

For in vitro diagnostic 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. The clinical interpretation of any staining or its absence should be complemented by morphological studies using proper controls and should be evaluated within the context of the patient's clinical history and other diagnostic tests by a qualified pathologist.

Synonym for antigen

Terminal complement complex, TCC (1); membrane attack complex, MAC.

Summary and explanation

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, polysacharride 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 (2, 3). For review of the complement system (2).

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 NaN_3.

Clone: aE11 (1). Isotype: IgG2a, kappa.

Mouse IgG concentration mg/L: See label on vial.

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 (4).

Precautions

1. For professional users.
2. This product contains sodium azide (NaN_3), 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.
4. Wear appropriate Personal Protective Equipment to avoid contact with eyes and skin.
5. Unused solution should be disposed of according to local, State and Federal regulations.

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 Dako Technical Services.

Specimen preparation

Paraffin sections: The antibody cannot be used for labeling of formalin-fixed, paraffin-embedded tissue sections.

Frozen sections and cell preparation: The antibody is recommended for use on acetone-fixed, frozen sections.

Staining procedure

Dilution: Monoclonal Mouse Anti-Human C5b-9, Code M0777, may be used at a dilution range of 1:25-1:50 when applied on acetone-fixed frozen 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 X0943, diluted to the same mouse IgG2a concentration as the primary antibody.

Visualization: Dako LSAB™+/HRP kit, Code K0679, and Dako EnVision™+/HRP kits, Codes K4004 and K4006, are recommended.

Performance characteristics

Normal tissues: The antibody labels follicular dendritic cells in germinal centres of secondary lymphoid follicles of reactive lymphoid tissues (5). 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 (6). Strong granular staining of deposits of TCC is observed in kidney biopsies (e.g. glomeruli and tubuli).

References

1. Mollnes TE, Lea T, Harboe M, Tschopp J. Monoclonal antibodies recognizing a neoantigen of poly(C9) detect the human terminal complement complex in tissue and plasma. Scand J Immunol 1985; 22:183-95.

2. Bhakdi S, Tranum-Jensen J. Membrane damage by complement. Biochim Biophys Acta 1983;737:343-72.
3. Bhakdi S, Käflein R, Halstensen TS, Hugo F, Preissner KT, Mollnes TE. Complement S-protein (vitronectin) is associated with cytolytic membrane-bound C5b-9 complexes. Clin Exp Immunol 1988;74:459-64.
4. Mollnes TE, Harboe M. Immunohistochemical detection of the membrane and fluid-phase terminal complement complexes C5b-9(m) and SC5b-9. Consequences for interpretation and terminology. Scand J Immunol 1987;26:381-6.
5. Halstensen TS, Mollnes TE, Brandtzaeg P. Terminal complement complex (TCC) and S-protein (vitronectin) on follicular dendritic cells in human lymphoid tissues. Immunology 1988;65:193-7.
6. Pettersen HB, Johnson E, Mollnes TE, Garred P. Synthesis of soluble C3 and C9 neoepitopes by human alveolar macrophages in vitro. Scand J Immunol 1988;28:431-4.

Explanation of symbols

REF	Catalogue number	2°C 8°C	Temperature limitation		Use by
IVD	In vitro diagnostic medical device	LOT	Batch code		Manufacturer
	Consult instructions for use	EC REP	Authorized representative in the European Community		

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