E06 MONOCLONAL ANTIBODY
(ANTI-OXIDIZED PL)

Background:
There is a growing body of evidence that oxidized lipids, particularly oxidized phospholipids (OxPL), play a crucial role in the development and pathology of inflammatory diseases and some infectious diseases. Atherosclerosis is widely considered to be a chronic inflammatory disease, and elevated plasma LDL is a major risk factor. Since oxidized LDL (OxLDL), with its associated OxPL, plays a major role in atherogenesis there has been a need for antibodies that specifically recognize OxLDL. The E06 monoclonal antibody meets this need and can discriminate between native LDL and OxLDL by binding to the phosphocholine headgroup of OxPL that is present in OxLDL but is absent from native LDL. In addition, E06 can detect OxPL in cells, tissues, membranes and lipoproteins in a variety of inflammatory settings.

E06 was extensively characterized in the laboratory of Dr. Joseph Witztum at the University of California at San Diego (UCSD). Avanti Polar Lipids, Inc.® is now producing the E06 murine monoclonal antibody under license from UCSD.

Antibody Information:
Antigen: Oxidized LDL.
Ig Class: Mouse IgM (kappa).
Specificity: E06 recognizes the phosphocholine headgroup of oxidized phospholipid that is present in oxidized LDL and PC-modified BSA. E06 does not bind to normal LDL or unoxidized PC.
Antibody Source: Monoclonal antibody from C57BL/6-derived hybridoma E06.
Production: In vitro cell culture.
Purification: Ultra filtration through 100 KDa cut-off filters.
Purity: ≥ 95%.
Formulation: E06 is provided as a sterile-filtered solution in phosphate buffered saline (PBS).
Mass and Concentration: Refer to Product Label.
Recommended Applications: ELISA, IHC and WB.
Storage conditions: Store undiluted at 4°C or lower.
Hazardous/Non-hazardous Components: This product contains no substances that, at their given concentration, are known to be hazardous to health. Therefore, there is no MSDS for this product.

Product use:
The E06 antibody has been used for the quantitation of oxidized LDL in, sandwich ELISA	extsuperscript{i}, direct ELISA	extsuperscript{ii}, competitive ELISA	extsuperscript{iii}, Immunohistochemistry (IHC)	extsuperscript{iv} and Western Blot analysis	extsuperscript{v}. The E06 antibody specifically binds to the PC headgroup of many oxidized phospholipids	extsuperscript{vi} and inhibits the binding of ox-LDL to macrophages.

A biotinylated form of E06 is used for enzyme-linked immunosorbent assay (ELISA) determination of ox-LDL in serum or plasma samples.
A TopFluor-conjugated E06 antibody is available for immunohistochemistry (IHC) including confocal microscopy.

Note:
After thawing, centrifuge this product at > 1,000 g for 5 minutes to collect any antibody solution that may be retained in the cap. We recommend long-term storage of E06 at -80°C. After it has been thawed for initial use, we recommend storing at 4°C. We do not recommend freeze-thawing or aliquoting.

FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.

Avanti No. | Description                  | No. of Assays |
------------|------------------------------|--------------|
330001S    | E06 monoclonal               | 100          |
330002S    | E06 monoclonal biotinylated | 100          |
330003S    | E06 monoclonal-TopFluor™    | 100          |

References:


text references...
APPLICATIONS:

1. Direct ELISA Protocol
For binding studies with the E06 antibody, 96-well polystyrene microtiteration plates were coated with 50µL of antigen (5µg/mL) in PBS containing 0.27 mM EDTA and 20 µM butylated hydroxytoluene overnight at 4°C. After washing, the plates were blocked with 2% BSA in PBS for 45 min at room temperature. The wells were washed four times with PBS containing 0.27 mM EDTA and 0.02% NaN₃. 50µL aliquots of the E06 antibody (5µg/mL) in PBS containing EDTA, NaN₃ and 3% BSA were added to each well and incubated overnight at 4°C. The amount of IgM bound to each well was quantitated with goat anti-mouse IgM-AP and a chemiluminescent substrate after 4h incubation at 4°C.

2. Competitive ELISA Protocol
To test the specificity of the E06 binding to antigens, microtiter plates were prepared as described in Protocol 1. A range of concentrations (0.1-20 µg/mL) of PC-BSA was included in the wells as a competition antigen along with 250 ng of E06 antibody. After incubation as described in Protocol 1, the amount of IgM specifically bound to each well was quantitated with goat anti-mouse IgM-AP and a chemiluminescent substrate after 4h incubation at 4°C.

3. Sandwich ELISA Protocol
The E06 antibody has been used as a detection antibody in a sandwich ELISA method. Microtiter wells were coated with the murine monoclonal antibody MB47 (5µg/mL) as a capture antibody to bind apo B-100. 1:50-diluted aliquots of plasma were added, followed by biotinylated E06 antibody and then streptavidin-linked HRP and a chemiluminescent substrate. In control wells, the monoclonal antibody MB24 was used as a detection antibody to quantitate total apo B-100 bound to the ELISA plate.

4. IHC Protocol
Atherosclerotic lesions in mouse or rabbit aortas have been probed with biotinylated E06 antibody. After fixation in formal sucrose (4% paraformaldehyde, 5% sucrose) the tissue was embedded in paraffin. Serial sections (8µm thick) were rehydrated and incubated with a 1:100 dilution of biotinylated E06 antibody. Color was developed with a streptavidin-alkaline phosphatase method.

5. Western Blot Protocol
The E06 antibody has been used to probe serum or LDL samples. Samples reduced with β-mercaptoethanol were run on 4-20% Gradient SDS-PAGE gels. Copper-oxidized LDL was included as positive control. The proteins were electrophoretically transferred to nitrocellulose membranes and were blocked with Super Block (Pierce Chemical Co.) for 45 minutes and then washed with TBS containing 0.05% Tween 20 (Polysorbate 20). The E06 antibody was diluted in TBS containing 3% BSA and 0.01% Tween 20, and the membrane was probed for 1h at room temperature. After washing the membranes for five to seven times to remove unbound E06 antibody, the bound antibody was detected with goat anti-mouse IgM conjugated to alkaline phosphatase. Alkaline phosphatase was visualized with nitroblue Tetrazolium and 5-bromo-4-chloro-3-indolyl phosphate as substrates (Biorad). Alkaline phosphatase activity can be detected by enhanced chemiluminescence (ECL) on X-ray film.