E06-Biotin 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. The E06 antibody specifically binds to the PC headgroup of many oxidized phospholipid and inhibits the binding of OxLDL to macrophages.

E06 has been 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-Biotin is provided as a sterile-filtered solution in tris-buffered saline (TBS containing 1% BSA).
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-Biotin is primarily used for sandwich ELISA determination of ox-LDL in serum or plasma samples. It can also be used in direct ELISA, competitive (IHC) and Western Blot analysis. For use in direct ELISA, make a 4 µg/mL working solution of 12 µg E06-Biotin in 3 mL of 1% BSA in PBS (1:62.5 dilution). Make a serial (1:1) dilution of this working solution to titrate the antibody. For competitive ELISA make a 2.5 µg/mL working solution (1:100 dilution).

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.

References:
APPLICATIONS:

1. Direct ELISA Protocol
For binding studies with the E06-Biotin antibody, 96-well Microfluor 1 white U bottom plates (Thermo 6905) were coated with 50µL of antigen (5µg/mL) in PBS containing 0.27 mM EDTA and overnight at 4°C. The wells were washed three times with PBS containing 0.27 mM EDTA. After washing, the plates were blocked with 3% BSA in PBS for 45 min at room temperature. 100µL aliquots of a serial dilution of the E06-Biotin antibody (0.78-400ng/well) in PBS containing EDTA and 1% BSA were added to each well and incubated 1hr at room temperature. The amount of IgM bound to each well was quantified with streptavidin-AP and a Lumi-Phos 530 substrate after 1hr incubation at room temperature.

2. Competitive ELISA Protocol
To test the specificity of the E06-Biotin binding to antigens, microtitration 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-Biotin antibody. After incubation as described in Protocol 1, the amount of IgM specifically bound to each well was quantified with streptavidin-AP and a chemiluminescent substrate after 1hr incubation at room temperature.

3. Sandwich ELISA Protocol
The E06-Biotin antibody has been used as a detection antibody for OxPL in a sandwich ELISA methodv. 96 well plates were coated with the murine monoclonal antibody MB47 (5µg/mL) as a capture antibody to bind apo B-100, the main protein of LDL and VLDL. After incubation as described in Protocol 1, the amount of IgM specifically bound to each well was quantified with streptavidin-AP and a chemiluminescent substrate after 1hr incubation at room temperature.

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

5. Western Blot Protocol
The E06-Biotin antibody has been used to probe serum or LDL samplesvi. Samples reduced with β-mercaptoethanol were run on 4-20%T 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-Biotin 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-Biotin antibody, the bound antibody was detected with streptavidin conjugated to alka- line phosphatase. Alkaline phosphatase was visualized with nitroblue Tetrazolium and 5-bromo-4-chloro-3-indolyl phos- phate as substrates (Biorad). Alkaline phosphatase activity can be detected by enhanced chemiluminescence (ECL) on X-ray film.

For more details: www.avantilipids.com
Phone: 800-227-0651
Email: lipidomics@avantilipids.com

Fig. 2. Sample Direct ELISA
Immobilized antigens, Cu-Ox LDL and LDL probed with E06-Biotin antibody followed by streptavidin-AP. E06-Biotin binding was measured as AP-dependent chemiluminescence.

Fig. 3. Sample Competitive ELISA
Immobilized antigen was Cu-Ox LDL, soluble competing antigen was PC-BSA probed with E06-Biotin antibody followed by streptavidin-AP. E06-Biotin binding was measured as AP-dependent chemiluminescence.