TECHNICAL DATA SHEET

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 phospholipids[iv] and inhibits the binding of ox-LDL to macrophages[iii].

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[v]. It can also be used in direct ELISA[iii], competitive (IHC)[iii] and Western Blot analysis[iii].
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.

Avanti No.	Description	No. of Assays
330002S	E06 monoclonal biotinylated	100

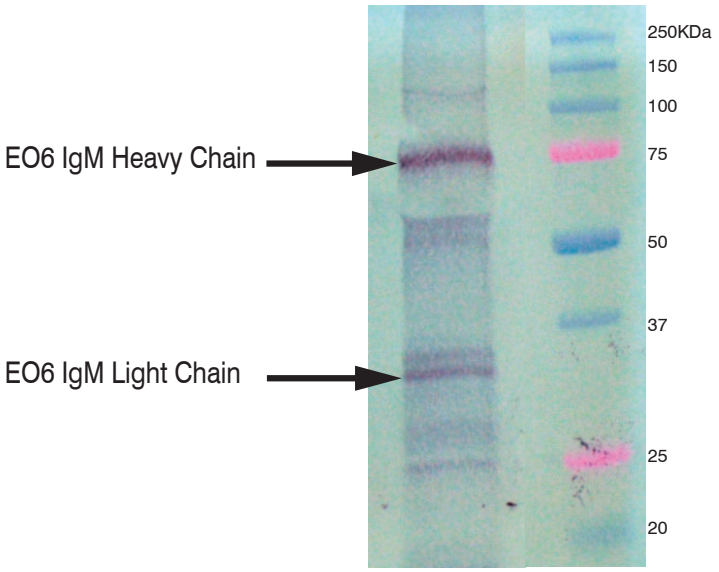

Fig. 1 Western blot of a SDS-PAGE gel of purified E06-Biotin antibody.
To detect biotin-labeled IgM protein subunits, the blot was probed with streptavidin-HRP using TMB as the substrate.

References:

[i] Palinski W, Hörkkö S, Miller E, Steinbrecher UP, Powell HC, Curtiss LK, Witztum JL. Cloning of monoclonal autoantibodies to epitopes of oxidized lipoproteins from apolipoprotein E-deficient mice. Demonstration of epitopes of oxidized low density lipoprotein in human plasma. *J Clin Invest.* 1996 98: 800-14.

[ii] Tsimikas S, Aikawa M, Miller FJ Jr, Miller ER, Torzewski M, Lentz SR, Bergmark C, Heistad DD, Libby P, Witztum JL. Increased plasma oxidized phospholipid:apolipoprotein B-100 ratio with concomitant depletion of oxidized phospholipids from atherosclerotic lesions after dietary lipid-lowering: a potential biomarker of early atherosclerosis regression. *Arterioscler Thromb Vasc Biol.* 2007 27: 175-81.

[iii] Hörkkö S, Bird DA, Miller E, Itabe H, Leitinger N, Subbanagounder G, Berliner JA, Friedman P, Dennis EA, Curtiss LK, Palinski W, Witztum JL. Monoclonal autoantibodies specific for oxidized phospholipids or oxidized phospholipid-protein adducts inhibit macrophage uptake of oxidized low-density lipoproteins. *J Clin Invest.* 1999 103: 117-28.

[iv] Friedman P, Hörkkö S, Steinberg D, Witztum JL, Dennis EA. Correlation of antiphospholipid antibody recognition with the structure of synthetic oxidized phospholipids. Importance of Schiff base formation and aldol condensation. *J Biol Chem.* 2002 Mar 1;277(9):7010-20.

[v] Tsimikas S, Bergmark C, Beyer RW, Patel R, Pattison J, Miller E, Juliano J, Witztum JL. Temporal increases in plasma markers of oxidized low-density lipoprotein strongly reflect the presence of acute coronary syndromes. *J Am Coll Cardiol.* 2003 41: 360-70.

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.

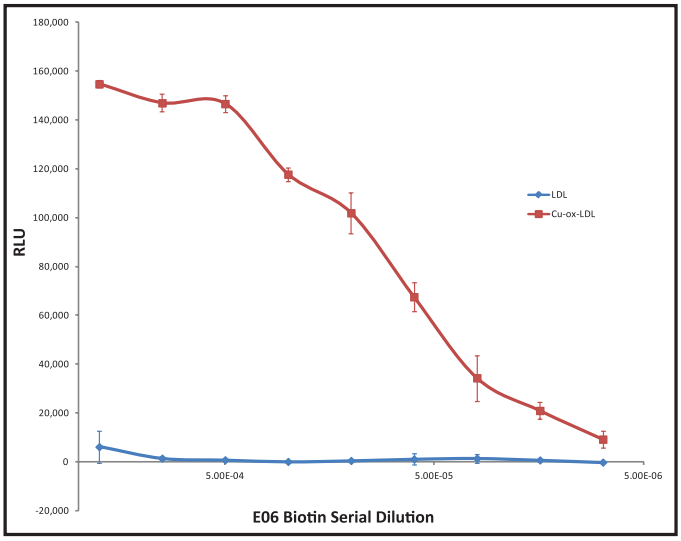

Fig. 2. Sample Direct ELISA

Immobilized antigens, Cu-Ox LDL and LDL probed with EO6-Biotin antibody followed by streptavidin-AP. E06-Biotin binding was measured as AP-dependent chemiluminescence.

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 method[ii]. 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. The plates were then blocked with PBS containing 3% BSA. 1:50-diluted aliquots of plasma were added and after a 1 hour incubation, the non-apo B100 containing material was washed off. E06-Biotin antibody was added at a 1:1,000 dilution and incubated for a further hour. After further washing, streptavidin-linked AP was added at a 1:5,000 dilution for 30 minutes and OxPL in the captured LDL was detected with a chemiluminescent substrate. In control wells, the monoclonal antibody MB24 was used as a detection antibody to quantify total apo B-100 bound to the ELISA plate.

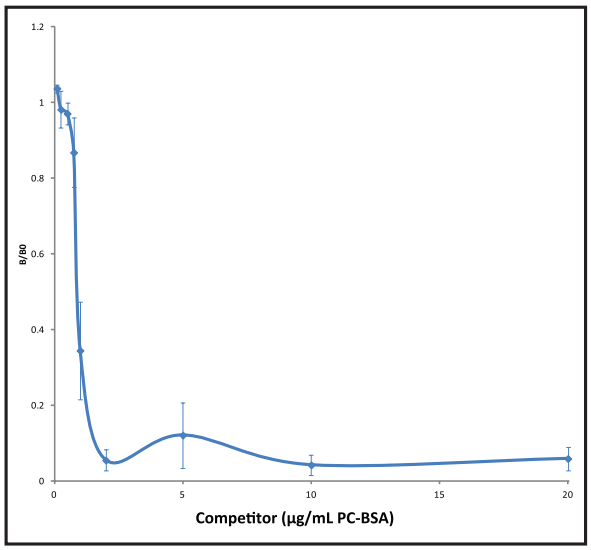

Fig. 3. Sample Competitive ELISA

Immobilized antigen was Cu-Ox LDL, soluble competing antigen was PC-BSA probed with EO6-Biotin antibody followed by streptavidin-AP. E06-Biotin binding was measured as AP-dependent chemiluminescence.

4. IHC Protocol

Atherosclerotic lesions in mouse or rabbit aortas have been probed with E06-Biotin 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 E06-Biotin antibody. Color was developed with a streptavidin-alkaline phosphatase method.

5. Western Blot Protocol

The E06-Biotin antibody has been used to probe serum or LDL samples[v]. 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 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.

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