TECHNICAL DATA SHEET

E06-TopFluor™ 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 Ox-LDL. 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[i]. 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 phospholipidsiv 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.

Avanti's new fluorescent probe - TopFluor™:

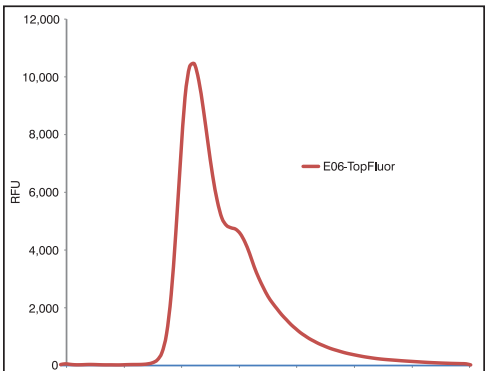


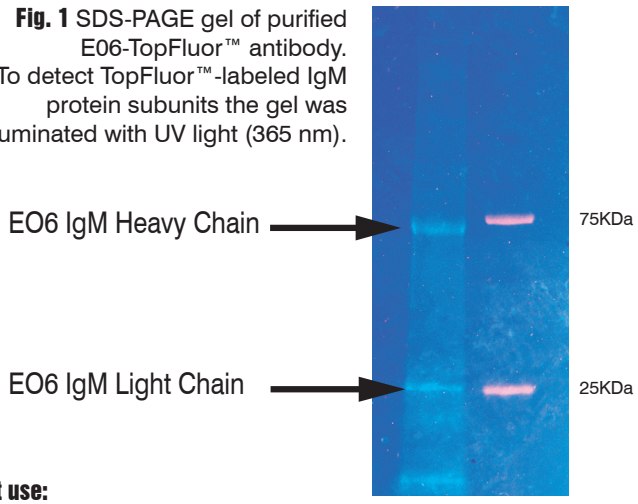
Fig. 2 The emission spectrum of E06-TopFluor™ between 395-751 nm was recorded with a Thermo Scientific NanoDrop 3300 fluorospectrometer. 2 μL of a 1 mg/mL solution was excited at 365 ± 10 nm. Maximum emission was detected at 511 nm.

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-TopFluor™ 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, flow cytometry, immunofluorescence and confocal microscopy.
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.

Avanti No.	Description	No. of Assays
330003S	E06 monoclonal-TopFluor™	100

Fig. 1 SDS-PAGE gel of purified E06-TopFluor™ antibody. To detect TopFluor™-labeled IgM protein subunits the gel was illuminated with UV light (365 nm).



Product use:

The E06-TopFluor™ is primarily use for direct ELISA[iii], immunefluorescence microscopy[v] and flow cytometry[v]. For use in direct ELISA, make a 4 μg/mL working solution of E06-TopFluor™ of 1% BSA in PBS. 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.

Note: After thawing, centrifuge this product at > 1,000 g for 5 minutes to collect any 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.

APPLICATIONS:

1. Direct ELISA Protocol

For binding studies with the E06-TopFluor™ antibody, 96-well Microfluor 1 black U bottom plates (Thermo 7005) 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-TopFluor™ 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 after 1hr incubation at room temperature with excitation (Ex) at 485 nm and emission (Em) recorded at 520 nm.

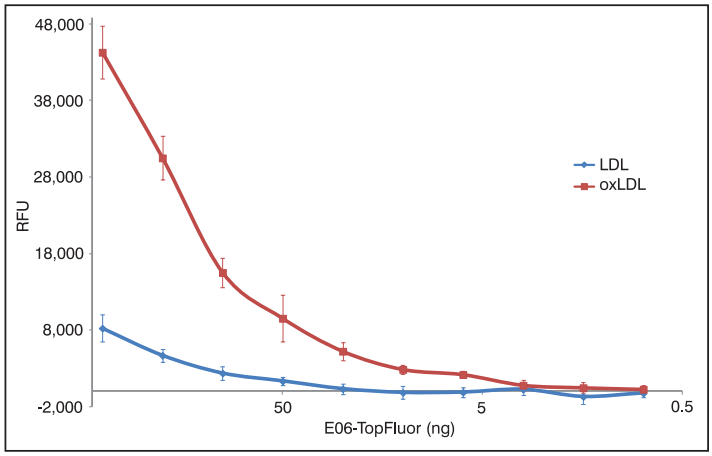


Fig. 3 Sample Direct ELISA

Immobilized antigens, Cu-Ox LDL and LDL probed with EO6-TopFluor™ antibody. E06-TopFluor™ binding was measured as fluorescence emission at 520 nm.

2. Competitive ELISA Protocol

To test the specificity of the E06-TopFluor™ binding to antigens, microtitration plates were prepared as described in Protocol 1. A range of concentrations (0.1-10 μg/mL) of PC-BSA was included in the wells as a competition antigen along with 250 ng of E06-TopFluor™ antibody. After incubation as described in Protocol 1, the amount of IgM specifically bound to each well was quantified with after 1hr incubation at room temperature with Ex at 485 nm and Em recorded at 520 nm.

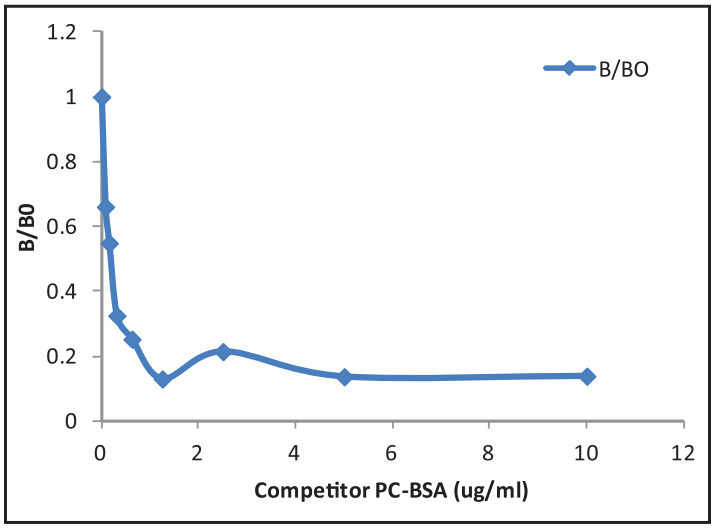


Fig. 4 Sample Competitive ELISA

Immobilized antigen was Cu-Ox LDL, soluble competing antigen was PC-BSA probed with EO6-TopFluor™ antibody. E06-TopFluor™ binding was measured as fluorescence emission at 520 nm.

3. Sandwich ELISA Protocol

The E06 antibody has been used as a detection antibody in a sandwich ELISA method[ii]. 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 E06-TopFluor™ antibody.

4. Immunofluorescence Microscopy Protocols

To probe apoptotic cells, J774A mouse monocyte/macrophages were plated on glass coverslips. Apoptosis was induced by serum starvation for 3h in 0.5% FBS and then incubation for 36-72 h with 1μg/mL PHAD in DMSO. The media was removed and the cells were blocked with PBS containing 1% BSA for 1h. The cells were then probed with E06-TopFluor (1:400) in PBS with 1% BSA for 1h. The cells were washed three times with PBS and the cover slips were mounted on slides and examined with a Zeiss Axiolab with a 40X/0.65 A-Plan objective an Ex 425/40 and Em 520/40 filters (Fig 5). In confocal microscopy, E06-TopFluor™ can be used in combination with Hoechst 33342 (Ex/Em = 346/497 nm) or DAPI (Ex/Em = 358/461 nm) nuclear staining and probes with fluorophores that emit at 578 nm and 670 nm using rhodamine and Cy5 filters.

5. Flow Cytometery Protocol

The E06-TopFluor™ antibody has been used to probe apoptotic cells and cells expressing oxidized lipids on their surface. After harvesting the cells, they were centrifuged and resuspended in PBS containing 5% FBS, 0.1% NaN3. The cells were probed with E06-TopFluor™ at a 1:400 dilution for 20 minutes at room temperature. After washing the cells three times with the above buffer to remove unbound E06-TopFluor™ antibody, the cells were fixed with 1% paraformaldehyde in PBS. The bound antibody was detected with the FL1 (Ex/Em = 488/530 nm) channel of a flow cytometer. If the paraformaldehyde fixation step is omitted, E06-TopFluor™ can be used in combination with probes with fluorophores that emit in the FL2 (Ex/Em = 488/578 nm) and FL3 (Ex/Em = 649/670 nm) channels.

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

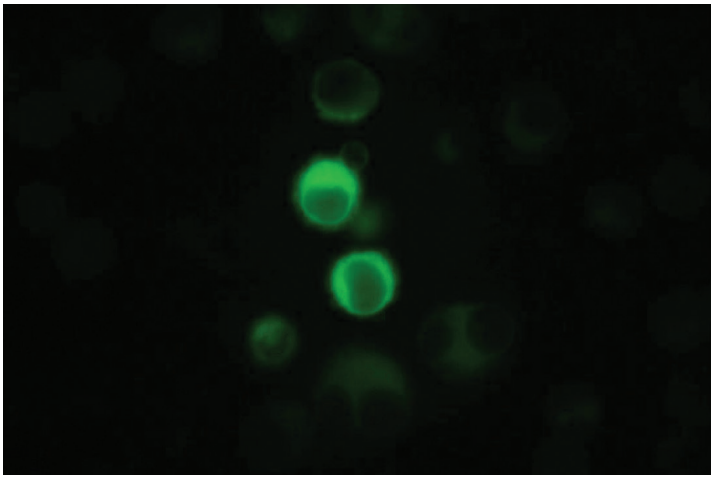


Fig. 5 Binding of E06-TopFluor™ to apoptotic cells.

J774A monocyte/macrophages cells were serum starved for 3h in 0.5% FBS and then incubated for 36 h with 1μg/mL PHAD in DMSO. The cells were blocked with PBS containing 1% BSA for 1h and were then probed with E06-TopFluor (1:400) in PBS with 1% BSA for 1h.

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] Chang MK, Bergmark C, Laurila A, Hörkkö S, Han KH, Friedman P, Dennis EA, Witztum JL. Monoclonal antibodies against oxidized low-density lipoprotein bind to apoptotic cells and inhibit their phagocytosis by elicited macrophages: evidence that oxidation-specific epitopes mediate macrophage recognition. *Proc Natl Acad Sci U S A.* 1999 96: 6353-8.

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