Technical Data Sheet

Avanti Lipid Snoopers® ELISA Strips

Introduction:

Understanding the molecular mechanism behind cellular processes often requires investigating numerous cellular interactions. In this regard, many methods have been developed for assaying and identifying unique protein-protein and protein-DNA interactions. However, one of the most abundant classes of molecules, lipids, has been widely excluded from such assessments suggesting a need for a screening method that allows for the rapid determination of protein-lipid interactions. Avanti Lipid Snoopers® ELISA strips are 8-well strips pre-coated with a single high-quality lipid species and are ideal for investigating protein-lipid interactions and characterizing and titrating lipid binding antibodies. The 8-well strip format allows for use as a standard 96-well plate, as well as allows individual 8-well strips to be removed from the frame providing convenience and maintaining integrity of unused wells when performing partial plate assays. The 8-well strips are available in optically clear, white, or black surfaces for use in colormetric, luminescent, and fluorescent assays, respectively. Avanti Lipid Snoopers® ELISA Strips allow multiple sample conditions to be probed simultaneously in a single plate making them a perfect complement to Avanti Lipid Snoopers® Nitrocellulose Strips.

Contents:

Each 8-well strip is packaged under argon and contains 1 nmol of high-quality pure lipid.

Note: The 18:1 CA ELISA Strips (Avanti Number 330621) contain 500pmol per well.

Storage:

Avanti Snoopers® ELISA Strips should be stored as packaged at 4°C until ready for use.

Stability:

Avanti Snoopers are stable for 6 months when stored as packaged at 4°C.

Experimental Protocol:

Materials Needed but Not Provided

• Washing Solution: Commonly used washing solutions include: Phosphate-Buffered Saline (PBS) (pH 7.4) or Tris-Buffered Saline (TBS) (pH 7.4)

• Blocking Solution: Commonly used blocking solutions include: 3% fatty acid free bovine serum albumin (BSA) or 0.3% gelatin in PBS or TBS

• Protein/Antibody Diluent: Antibodies and proteins of interest are most commonly diluted in 1X blocking solution.

General Procedure

• Remove 8-well strips from foil package and insert in the frame.

• Block: Add ~200µL of desired blocking agent to each well, cover wells with a plate cover, and incubate for 45 minutes - 1 hour at room temperature with gentle shaking.

• Wash: Decant blocking solution thoroughly (see Tips below) and wash the wells three times with ~200 µL of desired washing solution.*

• Incubate the ELISA Strip with Protein or Antibody of Interest: Add desired protein or antibody to each well in 1X blocking buffer (~50-100 µL of volume/well is recommended to ensure thorough coating of the well). Cover the wells with a plate cover and incubate for 1-2 hours at room temperature with gentle shaking.**

• Wash: Decant primary incubation solution thoroughly (see Tips below) and wash the wells three times with ~200 µL of desired washing solution.*

• Secondary Incubation: Following removal of the final wash solution, add a detector or secondary antibody to each well in 1X blocking buffer (~50-100 µL of volume/well is recommended to ensure thorough coating of the well). Cover the wells with a plate cover and incubate for 1-2 hours at room temperature with gentle shaking.*

• Wash: Decant secondary incubation solution thoroughly (see Tips below) and wash the wells three times with ~200 µL of desired washing solution.*

• Detection: Following removal of the final wash solution, detect the bound protein or antibody using a method of choice (i.e., colormetric, luminescent, or fluorescent etc.) and appropriate detection technique.

Notes:

In general, it is recommended to approach optimization and troubleshooting as you would with a standard ELISA.

* It may be necessary to optimize wash conditions by increasing the number and/or volume of washes in order to reduce background signal from non-specific interactions.

** It is essential to optimize the incubation time and concentration of the protein or antibody of interest, as well as the detector or secondary antibody, in order to reduce background caused from non-specific binding.

Tips:

Wash buffer and diluents containing detergent are not recommended for use with this product.

Solutions and washes may be easily removed by flicking the plate over a sink. The 8-well strips lock securely into frame and should not be lost during this procedure.

After the last wash in each round of washes, gently tap the plate on an absorbent surface in order to ensure that no washing solution is left in the wells. This will greatly reduce intra-assay variation.

Following the addition of the blocking solution, primary antibody, secondary antibody, and substrate, it may be necessary to gently agitate the plate containing the 8-well strips prior to incubation using the plate reader in order to ensure uniform distribution of the solution throughout the well. Alternatively, tap gently on the side of the plate to mix.

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

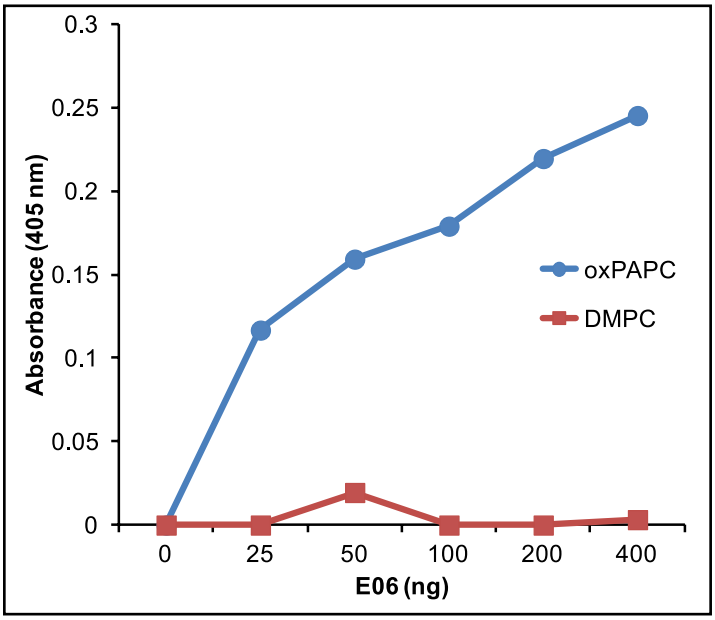

Figure 1: Colormetric (Absorbance) Assay using Optically Clear Lipid Snoopers® ELISA Strips Immobilized oxPAPC (2.5 nmol) and DMPC (2.5 nmol) were probed with E06 followed by a goat-anti-mouse IgM-HRP. E06 binding was measured as HRP-dependent absorbance following incubation with the ABTS® ELISA HRP substrate (KPL Inc., Gaithersburg, MD).

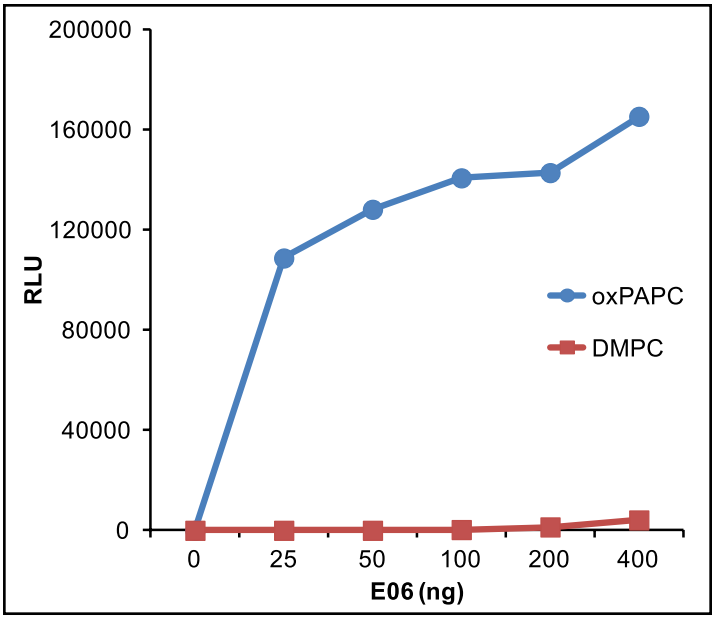

Figure 2: Luminescence Assay using White Lipid Snoopers® ELISA Strips Immobilized oxPAPC (2.5 nmol) and DMPC (2.5 nmol) were probed with E06 followed by a goat-anti-mouse IgM-AP. E06 binding was measured as AP-dependent luminescence following incubation with the Lumi-Phos 530 substrate (Lumigen, Southfield, MI).

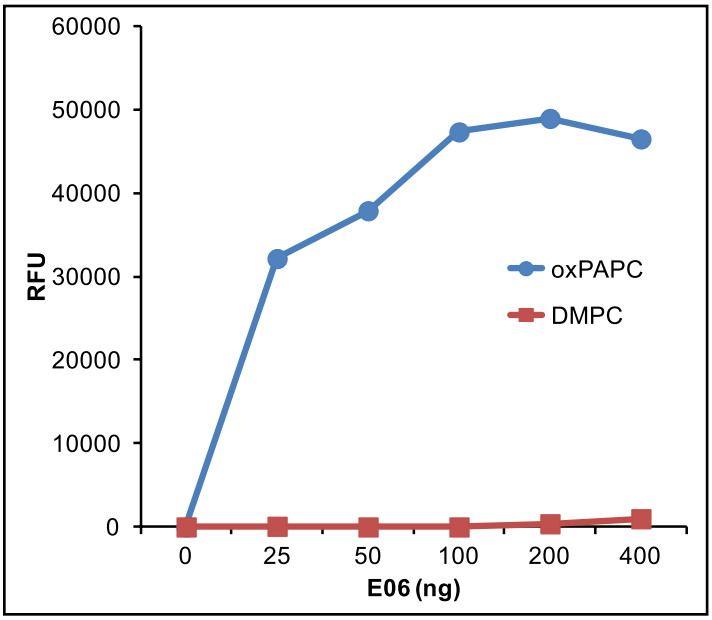

Figure 3: Fluorescence Assay using Black Lipid Snoopers® ELISA Strips Immobilized antigens, oxPAPC (2.5 nmol) and DMPC (2.5 nmol) probed with E06 antibody followed by a GAM IgM-HRP E06 binding was measured as HRP-dependent fluorescence.

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