



LipoProbes to Detect Membrane Protein Interactions

Optical Probes

Optically-labeled biological molecules (optical probes) are used in a host of applications, including binding kinetics, sub-cellular visualization, and target identification. While soluble proteins and chemicals are commonly used as optical probes, integral membrane proteins, such as GPCRs, have been difficult to use as probes because of their requirement for an intact lipid bilayer. Integral Molecular's LipoProbe enables labeled membrane proteins to be used in the mobile phase for probing molecular targets such as arrays, beads, or cells.

The LipoProbe

The LipoProbe utilizes Integral Molecular's Lipoparticle technology, in which membrane proteins are incorporated into nanoscale cell membrane vehicles. Lipoparticles can also co-incorporate a variety of optical reporters, including fluorescent proteins, enzymes, and small molecule dyes. The resulting reagent is the LipoProbe, which combines user-defined membrane proteins with optical reporters. Because of their size (~150 nm), LipoProbes can be used as mobile-phase reagents to probe membrane protein interactions. Their high concentrations of membrane proteins and reporter molecules ensures sensitive target detection. Furthermore, multiple reporters and modifying molecules can be combined for greater specificity or multiplexed detection. The flexibility of the LipoProbe permits a wide range of extracellular and cell-free applications that are suited to a variety of detection modalities, including flow cytometry, microscopy, microplates, and microarrays.

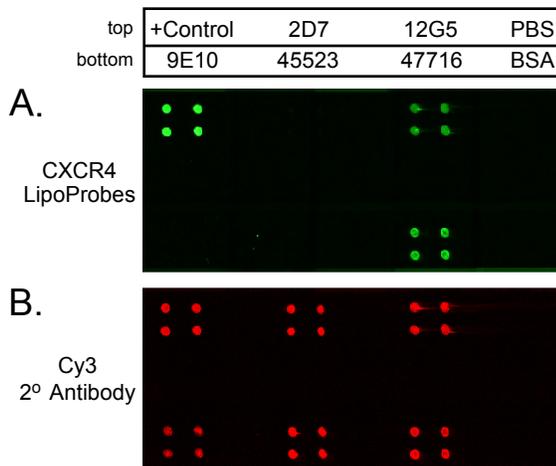


Figure 1. A. An antibody microarray was spotted on glass slides and probed with green fluorescent LipoProbes containing the GPCR CXCR4. **B.** A Cy3-labeled (red) anti-IgG antibody was used to verify the integrity of each antibody spot in the array.

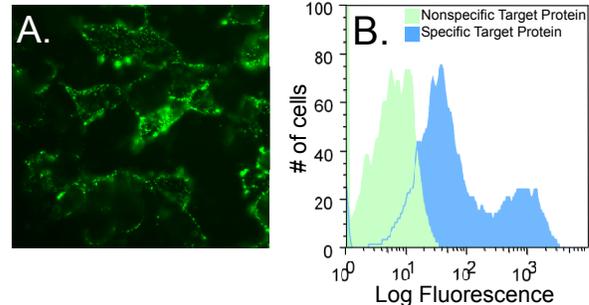


Figure 2. A. HEK-293 cells expressing the ASLV-A Envelope protein were probed with green fluorescent LipoProbes containing the viral cell surface receptor Tva. **B.** Using flow cytometry, LipoProbes were capable of differentiating cells expressing ASLV-A from those expressing a non-specific Envelope protein.

Technical Description

LipoProbes have been used as detection reagents in a number of applications. Figure 1 demonstrates the ability to probe antibody microarrays using LipoProbes incorporating both the chemokine receptor CXCR4 (a GPCR) and a green fluorescent reporter. LipoProbes robustly detected quadruplicate spots of antibodies with known specificity for CXCR4 (Mabs 12G5 and 47716). Spots of positive control antibodies with specificity for another tag on the Lipoparticle surface were also detected, while non-specific antibodies and controls were not. The integrity of the antibody spots was verified using a Cy3-labeled anti-IgG antibody in the same experiment (bottom panel). LipoProbes have also been used to detect proteins expressed on cell surfaces, including membrane protein-membrane protein interactions. Green fluorescent LipoProbes incorporating Tva, a cell membrane protein that is used as a receptor by some viral pathogens, were used to probe cells expressing the Envelope protein of the Avian Sarcoma-Leukosis Virus (ASLV-A). Fluorescent microscopy demonstrates the localization of LipoProbes on the surface of cells expressing the ASLV-A viral protein (Figure 2). When analyzed by flow cytometry, LipoProbes could quantitatively differentiate between cells that were expressing the Tva receptor, and those that were not. LipoProbes thus make it possible to detect the interactions of membrane proteins for novel applications.

Contact Us

LipoProbes are produced with customized membrane proteins, and are validated for use with specific applications. Evaluation materials are available for new applications. For more information contact us at:

215.966.6061

info@integralmolecular.com

www.integralmolecular.com