Echelon Biosciences Inc.

Lipid Coated Microparticles

Lipid microparticles are beads with attached phospholipids where the phospholipid headgroups are exposed and available for biological interactions. Two lipids and two fluorescent dyes are available. Phosphatidylserine (PS) is an anionic, intracellular phospholipid component of the cell membrane and is involved in cell signaling. Phosphatidylcholine (PC) is primarily an extracellular phospholipid component of cell membranes and is a key structural lipid. Lipid coated microparticles are intended for use in apoptosis and phagocytosis studies.

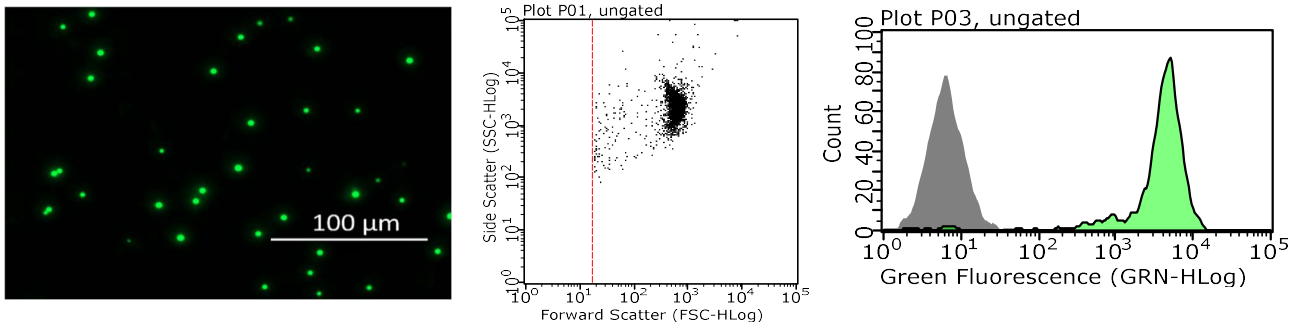
Product #	Lipid	Dye
P-B1PC	Phosphatidylcholine (PC)	NBD
P-B1PCr	Phosphatidylcholine (PC)	pHrodo™ Red
P-B1PCnf	Phosphatidylcholine (PC)	Unlabeled
P-B1PCPS	PC to PS ratio of 75:20	NBD
P-B1PS	Phosphatidylserine (PS)	NBD
P-B1PSr	Phosphatidylserine (PS)	pHrodo™ Red
P-B1PSnf	Phoshpatidylserine (PS)	Unlabeled

pHrodo is the trademark of ThermoFisher Scientific

Formulation: Microparticles are in PBS, pH 7.4., 0.02% sodium azide.

Concentration: See certificate of analysis.

Storage: Store product at 2-4 °C. **Do not freeze.**



Left: PS Lipid Microparticles imaged at 470 nm excitation/525 emission with GFP filter at 40X magnification.
Middle: Flow cytometry dot plot of PS Lipid Microparticles. The x-axis is relative size and the y-axis is microparticle complexity.
Right: Flow histogram contrasting PS Lipid Microparticles with non-fluorescent microparticles using a Millipore Guava 8HT. The x-axis represents fluorescence intensity.

Technical Notes

1. Total lipid concentration is approximately 1 µmol per 1 mg microparticles.
2. Fluorescent containing microparticles contain trace amounts of a fluorescent lipid.
    a. NBD has maximal excitation/emission at approximately 460/540 nm.
    b. pHrodo™ Red has maximal excitation/emission at approximately 560/590 nm.
3. The microparticle is comprised of a silica core and is 3 µm.
4. Lipid coated microparticles contain 0.02% sodium azide. If your experiments are sensitive to sodium azide, remove it before use. eg. 1 x PBS buffer, pH 7.4, followed by centrifugation @10,000 xg for 10 minutes.

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5. Centrifuge the microparticles at 10,000 x g or lower. Higher centrifugation may damage the microparticles.
6. AnnexinV demonstrates binding to the PS containing microparticles. AnnexinV does not bind the PC containing microparticles.

Preparation Notes

We provide the following section as a starting point, and strongly encourage researchers to consult the scientific literature and to conduct optimization experiments to establish the most favorable conditions for their experiments. Other buffers and conditions may show improved results.

1. Microparticles can aggregate with storage. Before each use, sonicate for 5-10 minutes in a water bath sonicator followed by 1 minute of vortexing to ensure uniform suspension.
2. Pre-treating the microparticles with a blocking buffer reduced non-specific binding of AnnexinV to PC coated microparticles and appears to reduce non-specific uptake of the microparticles in RAW 264.7 macrophages. eg. 10 mM HEPES, 140 mM NaCl, 2.5 mM CaCl2, 5% BSA and 0.25% Tween20, followed by centrifugation @10,000 X g for 10 minutes.
3. Dilute the microparticles with your preferred assay buffer to roughly 2.5 - 1 x 10^6 microparticles / mL. This microparticle suspension should be further diluted 1:4 - 1:10 in cell media before addition to the cells. This is to dilute any item contained in the assay buffer, such as detergent, that may negatively impact the cells. We added microparticles to the RAW 264.7 macrophages at a 1:4 ratio in cell media. This was roughly 0.25 x10^6 microparticles in 1 mL cell media.
4. After incubation with microparticles, we suggest removing non-ingested microparticles with washing. This can be followed by a quenching step.
    a. For NBD containing microparticles, NBD can be quenched with 0.4% trypan blue.

References

1. Neil Paterson, Tim Lämmermann (2022) Macrophage network dynamics depend on haptokinesis for optimal local surveillance eLife 11:e75354
2. Nabil Rabhi, Kathleen Desevin (2022) Obesity-induced senescent macrophages activate a fibrotic transcriptional program in adipocyte progenitors Life Science Alliance DOI: 10.26508/lsa.202101286
3. Brando Cieniewicz, Ankit Bhatta (2023) Chimeric TIM-4 receptor-modified T cells targeting phosphatidylserine mediates both cytotoxic anti-tumor responses and phagocytic uptake of tumor-associated antigen for T cell cross-presentation Molecular Therapy Vol. 31 No 7
4. Rachel Grazda, Allison N. Seyfried (2023) Impaired inflammation resolution in murine bone marrow failure is rescued by Resolvin E1 treatment bioRxiv preprint https://doi.org/10.1101/2023.02.15.528688

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