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Technical Data Sheet

For research use only

Not intended or approved for
diagnostic or therapeutic use.

Product Name: Lipid Coated Beads

P-B000 - Control PIP Beads TM	P-B035a - PI(3,5)P ₂ PIP Beads TM	P-BLBPA - LBPA Beads
P-B001 - PtdIns PIP Beads TM	P-B045a - PI(4,5)P ₂ PIP Beads TM	S-6100 - Sphingosine Beads
P-B003a - PI(3)P PIP Beads TM	P-B345a - PI(3,4,5)P ₃ PIP Beads TM	S-6110 - S1P Beads
P-B004a - PI(4)P PIP Beads TM	Q-B0145 - Ins(1,4,5)P ₃ Beads	P-B0SM - Sphingomyelin Beads
P-B005a - PI(5)P PIP Beads TM	Q-B1345 - Ins(1,3,4,5)P ₄ Beads	P-BCer - Ceramide Beads
P-B034a - PI(3,4)P ₂ PIP Beads TM	L-6101 - LPA Beads	P-BCLP- Cardiolipin Beads
P-BPEth- PEth Beads		

Contains:

- One mL of agarose beads (50% slurry in 1X PBS buffer) contains 10 nanomoles of bound lipid.
- Each order of lipid coated beads include 200 μ L of control beads.

Product Storage: Store at 2-8°C. Product is moisture and light sensitive. **Do not freeze.**

Description & Suggested Usage: Designed to study protein-lipid interactions in lipid-protein pull-down experiments. Possible uses are to test for specific lipid-protein binding interactions from a variety of protein sources. These include purified protein solutions, cell lysate and radiolabeled *in vitro* translation proteins.

References: Rao, V. et al. Expression Cloning of Protein Targets for 3-phosphorylated phosphoinositides. *J. Biol. Chem.* **274**(53): 37893-37900 (1999)

Yuya Kunisaki, Akihiko Nishikimi, Yoshihiko Tanaka, et al. DOCK2 is a Rac activator that regulates motility and polarity during neutrophil chemotaxis. *J. Cell Biol.*, Aug 2006; 174: 647 - 652.

Naava Naslavsky, Juliati Rahajeng, Sylvie Chenavas, Paul L. Sorgen, and Steve Caplan. EHD1 and Eps15 Interact with Phosphatidylinositols via Their Eps15 Homology Domains. *J. Biol. Chem.*, Jun 2007; 282: 16612-16622.

Lipid Bead - Protein Pull-down Protocol

- Use 50-100 μ L of beads for each pull-down assay. (Note: 50 μ L of bead = 100 μ L of 50% slurry)
- Pellet beads by centrifugation at 1,000 rpm or lower. Higher speeds can crush the beads. Resuspend the beads in an equal volume of wash/binding buffer.

1. Add 1-10 μ g of protein, diluted in binding buffer, to 50 – 100 μ L of beads. When using cell lysate, avoid harsh lysis procedures and ionic detergents which may interfere with lipid binding.
2. Incubate protein solution and beads for 1-3 hours. The incubation can be done at room temperature or at 4° C, depending on the stability of your protein.
3. Wash beads 2-5 times with 10X excess of wash/binding buffer, pelleting beads between washes and carefully removing the wash solution to avoid losing beads.
4. To elute proteins, add an equal volume of 2X Laemmli sample buffer to beads and heat to 95 °C for several minutes. Proteins can be separated by SDS-PAGE and analyzed by Coomassie blue staining of the gel, protein transfer and immunoblotting to detect proteins of interest, or autoradiography.

Wash/Binding Buffer

10mM HEPES, pH 7.4
150mM NaCl
0.25% Igepal

*Note: We suggest 0.25% Igepal as a starting concentration. If the protein is sticking nonspecifically to control beads, try increasing the detergent concentration to 0.5%.

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