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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™

P-B001 - PtdIns PIP Beads™

P-B003a - PI(3)P PIP Beads™

P-B004a - PI(4)P PIP Beads™

P-B005a - PI(5)P PIP Beads™

P-B034a - PI(3,4)P<sub>2</sub> PIP Beads™

P-B035a - PI(3,5)P<sub>2</sub> PIP Beads™

P-B045a - PI(4,5)P<sub>2</sub> PIP Beads™

P-B345a - PI(3,4,5)P<sub>3</sub> PIP Beads™

Q-B0145 - Ins(1,4,5)P<sub>3</sub> Beads

Q-B1345 - Ins(1,3,4,5)P<sub>4</sub> Beads

L-6101 - LPA Beads

P-BLBPA - LBPA Beads

S-6100 - Sphingosine Beads

S-6110 - S1P Beads

P-B0SM - Sphingomyelin Beads

P-BCer - Ceramide 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 µL 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 50-100µL of beads for each pull-down assay. (Note: 50µL of bead = 100µL 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 µg of protein, diluted in binding buffer, to 50 - 100µL 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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