Lipid Coated Beads

Lipid Beads™ are designed for protein pull-down experiments. Possible uses are to test for lipid binding in a solution of purified protein, in a cell lysate, or to test binding of radiolabeled *in vitro* translation products.

Lipid Bead	Catalog #
Control	P-B000
PtdIns	P-B001
PI(3)P	P-B003a
PI(4)P	P-B004a
PI(5)P	P-B005a
PI(3,4)P2	P-B034a
PI(3,5)P2	P-B035a
PI(4,5)P2	P-B045a
PI(3,4,5)P3	P-B345a
Cardiolipin	P-BCLP
Ceramide 1-phosphate	P-BC1P
Ceramide	P-BCer
LBPA	P-BLBPA

Lipid Bead	Catalog #
Lactosyl Ceramide	P-B0LC
Phosphatidic Acid	P-B0PA
Phosphatidylcholine	P-B0PC
Phosphatidylethanolamine	P-B0PE
Phosphatidylserine	P-B0PS
Sphingomyelin	P-B0SM
Phosphatidylethanol	P-BPEth
Cholesterol	P-BChl
Lysophosphatidic Acid	L-6101
Ins(1,3,4,5)P4	Q-B1345
Sphingosine	S-6100
Sphingosine 1-phosphate	S-6110
PIP Beads Sample Pack	P-B00S
Sphingo Beads Sample Pack	P-B00SS

Lipid Bead™ Assay Notes

1. Lipid Coated Beads are available in 1 mL and 0.1 mL sizes and come as a 50% slurry in 1X PBS Buffer.
2. An order of 1 mL comes with 200 µL of Control Beads. Control Beads are also available for purchase and their use is suggested for analysis.
3. Store at 2-8°C. Product is temperature and light sensitive. Do not freeze.
4. Centrifuge the beads at 1,000 x g or lower. Do not centrifuge the beads at high speed as this can crush the beads.
5. There is a total of 10 nmoles of bound lipid per 1 mL of beads.
6. The beads range from 45 – 165 micrometers in diameter.
7. Use 50-100 µL of 50 % slurry lipid coated beads and control beads for each protein.
8. See table 1 for a list of positive control proteins

Lipid Bead™ - Protein Pull-down Protocol

We provide the following protocol as a guide, and strongly encourage researchers to consult the scientific literature and conduct optimization experiments to establish the most favorable conditions for their protein of interest.

1. Mix beads well (do not vortex). Transfer 50 – 100 µL of 50% bead slurry to a 0.5 – 1.5 mL tube.
2. Pellet beads by centrifugation at 1,000 x g or lower. Carefully remove supernatant to avoid losing beads.
3. Wash beads with 10X excess of the wash buffer. Incubate wash 1-2 min with gentle mixing. Repeat wash steps two times.
4. Carefully remove wash supernatant and resuspend the beads in binding buffer containing your protein.
    a. For purified proteins, use 1-10 µg of protein diluted in enough binding buffer to form a 50% lipid bead slurry.
    b. For cell lysates, extracts, subcellular fractions: use approximately 1 mg of total protein to 1 mL

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binding buffer. Add the entire 1mL of prepared sample to your washed beads.

5. Incubate the protein-bead solution for 1-3 hours. The incubation can be done at room temperature or at 4° C, depending on the stability of your protein. Continuous motion is required to keep beads in suspension.
6. Pellet the beads and carefully remove supernatant. If needed, the supernatant can be used to monitor unbound protein.
7. Wash beads with 10X excess of the wash buffer. Incubate wash 1-2 min with gentle mixing. Repeat wash steps two to five times. If you choose to use BSA in your binding buffer make sure it is washed out well before moving to the next step.
8. Following the last wash, elute bound proteins by adding an equal volume of 2X Laemmli sample buffer (or similar) to beads and heat to 70 °C for 10 minutes.
9. Following heating, pellet the beads and remove the supernatant. Store supernatant at 4°C until analysis. Discard beads.
10. 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.

Suggested Buffer Conditions

The buffers below are a good starting point for testing lipid protein interactions. Other buffers may show improved binding with your protein of interest. Protein stabilizers, such as 0.1-1% BSA, can stabilize some proteins and reduce non-specific lipid-protein interactions. Non-ionic detergents, such as Igepal and Tween 20, can reduce non-specific lipid interactions and reduce background binding to control beads.

Buffer System 1

Wash/Binding Buffer: 10 mM HEPES, pH 7.4, 150 mM NaCl, 0.25% Igepal

If the protein is sticking nonspecifically to Control Beads, try increasing the detergent concentration to 0.5% or higher.

Buffer System 2

Binding Buffer: 0.1-1% BSA in PBS with 0.05-0.1% Tween 20, pH 6.8-7.4

Wash Buffer: PBS with 0.05-1% tween 20, pH 6.8-7.4

Table 1: Positive Control Proteins: Bead - protein pairs for use as positive controls in your experiments.

Lipid binding protein	Catalog #	Suggested Buffer Condition	Lipid Bead (Catalog #)
MultiPIP Grip	G-9901	1, 2	PI(3,4)P2 Bead (P-B034a) PI(3,4,5)P3 Bead (P-B345a)
PI(3)P Grip	G-0302	2	PI(3)P Bead (P-B003a)
PI(4)P Antibody	Z-P004	2	PI(4)P Bead (P-B004a)
PI(4,5)P2 Grip	G-4501	1, 2	PI(4,5)P2 Bead (P-B045a)
PI(3,5)P2 Grip	G-3501	2	PI(3,5)P2 Bead (P-B035a)
PI(3,4,5)P3 Grip (Grp1-PH)	G-3901	1, 2	PI(3,4,5)P3 Bead, (P-B345a) Ins(1,3,4,5)P4 Bead (Q-B1345)
Lysophosphatidic Acid Antibody	Z-P200	2	Lysophosphatidic Acid Bead (L-6101)

Table 2: Troubleshooting Guide

Issue	Suggestion
No binding	• Increase protein concentration • Increase incubation time

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	• Add a protein stabilizer such as BSA • Protein has lost activity
More than one band	• Increase detergent concentration in wash/Binding Buffer • Increase number of washes • Add longer incubation steps per each wash
Binds Control bead	• Increase detergent concentration in wash/Binding Buffer • Increase number of washes • Add longer incubation steps per each wash

Preparation of Cell Lysate / Protein (Process may vary by cell type / source)
This is a general method for when using cell lysate. Harsh lysis conditions should be avoided as they may interfere with lipid structure and binding. Non-ionic detergents such as Igepal, NP40, and Triton X-100 are less harsh than ionic detergents, non-denaturing, and used for solubilizing and purification of membrane protein complexes.

1. Isolate and centrifuge cells.
2. Lyse the pellet. (See below for lysis buffer suggestions)
3. Vortex and centrifuge to prepare clarified samples.

Lysis Buffer
Salts (10-20 mM HEPES, pH 7.4, 100-150 mM NaCl)
Non-ionic detergents* (such as 0.1 – 2% Igepal)
Divalent cations (0-10 mM EDTA and/or EGTA)
Protease inhibitors (cOmplete mini protease tablet, Na orthovanadate, Na fluoride.)

Statement

The protein-pull down protocol has been tested with the beads and proteins contained in table 1. Protein-lipid interactions determined with Echelon's lipid coated bead products may vary from the binding interactions determined in other methods. We recommend researchers use a variety of methods to fully characterize the lipid binding preference of a particular protein. Echelon has several innovative products useful for determining protein-lipid interactions. These products include lipid overlay products such as PIP strips (P-6001), stabilized liposomes (PolyPIPosomes™ e.g. Y-P039) and PIP-Plates™ (e.g. H-6300). Please contact our technical service representatives by email at echelon@echelon-inc.com; or by phone, toll-free 866-588-0455, with any questions or to provide feedback and suggestions.

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