Echelon Biosciences Inc.

Lipid Coated Beads

Lipid Beads™ are designed for use in protein pull-down experiments. Possible uses are to test for PIPn 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(1,4,5)P3	Q-B0145
Ins(1,3,4,5)P4	Q-B1345
Sphingosine	S-6100
Sphingosine 1-phosphate	S-6110

Lipid Bead™ 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 are 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 beads for each pull-down assay. (Note: 50 µL of bead = 100 µL of 50% slurry)

Lipid Bead™ - Protein Pull-down Protocol

1. Pellet beads by centrifugation at 1,000 x g or lower.
2. Remove the supernatant and resuspend the beads in an equal volume of wash-binding buffer. Add 1-10 µg of protein, diluted in binding buffer, to 50 – 100 µL of beads.
   - When using cell lysate, avoid harsh lysis conditions which may interfere with lipid structure and binding.
3. 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.
4. Pellet the beads and remove the supernatant.
   - Carefully remove the wash solution to avoid losing beads.
5. Wash the beads with 10X excess of the wash/binding buffer.
6. Repeat wash steps 4 and 5, two to five times.
7. Following the last wash, elute bound proteins by adding an equal volume of 2X Laemmli sample buffer (or similar) to beads and heat to 95 °C for 5-10 minutes.
8. Following heating, pellet the beads, remove the supernatant, and store supernatant at 4°C until analysis.

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

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% or higher.

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