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Product Protocol

For research use only
Not intended or approved for diagnostic or therapeutic use.

PIP Beads™

Phosphoinositide-coated agarose beads

Basic PIP Beads™ Protocol

PIP 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.

One mL of packed agarose beads (supplied as 50% slurry in PBS buffer) contains 10 nanomoles of bound PIPn. Use 50-100µL of beads for each pull-down assay.

Pellet beads by centrifugation at 1000rpm or lower. High speeds can crush the beads. Resuspend the beads in an equal volume of wash/binding buffer.

Add 1-10 µg of protein, diluted in binding buffer, to 100µL of beads. When using cell lysate, avoid harsh lysis procedures and ionic detergents which may interfere with lipid structure.

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.

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.

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
0.25% NP-40
150mM NaCl

We suggest 0.25% NP-40 as a starting concentration. If the protein is sticking nonspecifically to control (no PIPn) beads , try increasing the detergent concentration to 0.5%.