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Technical Data Sheet

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

Product Name: Neo Beads

Product Number: **P-B999**

Quantity: 20 mg

How Supplied: Dried microsphere solids

Storage: Product is stable at room temperature for up to one year when stored dry, in water, or in methanol/water solutions.

Binding Capacity: Each milligram of beads will bind at least 1 nmole phosphoinositide.

Suggested Use: Selective purification/immobilization of phosphoinositides from biological samples. Phosphoinositides can be recovered from the beads using fresh 2 M aqueous triethylamine bicarbonate, chloroform, methanol (3:2:6; v/v).[4] See attached protocol for details.

References:

1. Schacht, J., Purification of polyphosphoinositides by chromatography on immobilized neomycin, *J Lipid Res, 19*, 1063 (1978).

2. Klyashchitsky, B. A., Mezhova, I. V., Krasnopolsky Yu, M., and Shvets, V. I., Preparative isolation of polyphosphoinositides and other anionic phospholipids from natural sources using chromatography on adsorbents containing primary amino groups, *Biotechnol Appl Biochem, 14*, 284 (1991).

3. Meijer, H. J., Berrie, C. P., Iurisci, C., Divecha, N., Musgrave, A., and Munnik, T., Identification of a new polyphosphoinositide in plants, phosphatidylinositol 5-monophosphate (PtdIns5P), and its accumulation upon osmotic stress, *Biochem J, 360*, 491 (2001).

4. Sbrissa, D., Ikonomov, O. C., Deeb, R., and Shisheva, A., Phosphatidylinositol 5-phosphate biosynthesis is linked to PIKfyve and is involved in osmotic response pathway in mammalian cells, *J Biol Chem, 277*, 47276 (2002).

5. A. Grey, H. Olsson, I.H. Batty, L. Priganica, C.P. Downes: Nonradioactive methods for the assay of phosphoinositide 3-kinases and phosphoinositide phosphates and selective detection of signaling lipids in cell and tissue extracts. *Analytical Biochemistry* 313 234-245 (2003).

Lipid Extraction Protocol: *Extraction of phosphoinositides from cells*

The procedure as verified with 5 x 10^6 cells (75 cm^2 flask at 60% confluency). Larger or smaller amounts of cells require proportional adjustments of volumes. The amount of cells necessary for each phosphoinositide quantification needs to be determined for each cell type.

Solutions for Extraction:

0.5 M TCA: For 50 mL, dissolve 4.08 g TCA (Trichloroacetic Acid) in dH_2O and bring volume to 50 mL.

5% TCA with 1 mM EDTA: For 50 mL, dissolve 2.5 g TCA in dH_2O, and add 100 µL 0.5 M EDTA, and bring volume to 50 mL with dH_2O.

MeOH: $CHCl_3$ (2:1): For 60 mL, add 40 mL MeOH to 20 mL $CHCl_3$.

Elution Buffer: For 44 mL add 24 mL MeOH and 12 mL 2 M TEAB (Triethylamine Buffer) together first, then slowly add 8 mL $CHCl_3$. The three solutions are not soluble when mixed simultaneously.

Procedure:

1. Collect cells: Aspirate media from cells. Add 4 mL cold 0.5 M TCA. Incubate on ice for 5 min. Scrape cells and transfer into 15 mL tube. Centrifuge at 1,500 rpm for 5 min at 4˚C. Discard supernatant.

2. Wash pellet: Add 3 mL 5% TCA/1 mM EDTA to cell pellet. Vortex. Centrifuge at 1,500 rpm for 5 min at 4˚C. Discard supernatant. Repeat wash one more time.

3. Extract neutral lipids: Add 3 mL MeOH: $CHCl_3$ (2:1) to pellet. Incubate 10 min at room temperature. Vortex 3 times during incubation. Centrifuge at 1,500 rpm for 5 min at 4˚C. Discard supernatant.

4. NeoBeads: Add 3 mL MeOH: $CHCl_3$ (2:1) and 1-5 mg Neobeads to pellet and incubate 10 min at room temperature. Vortex 3 times during incubation. Centrifuge at 1,500 rpm for 5 min at 4 ºC. Discard supernatant.

5. Binding PIPs: Add 3 mL of distilled water to pellet. Incubate 20 min at room temperate with constant agitation. Centrifuge at 1,500 rpm for 5 min at 4 ºC. Discard supernatant.

6. Eluting PIPs: Add 1 mL of Elution Buffer to pellet. Incubate 10 min at room temperate with constant agitation. Centrifuge at 1,500 rpm for 5 min at 4 ºC. Transfer supernatant to a new vial and dry with Nitrogen stream of Speed Vac. Repeat elution step one more time. Combine extracted lipids together and dry down.

Note: Dry lipid extracts can be stored at -20 ºC.