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Technical Data Sheet

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

Product Name:
TMR-labeled Carrier 2
Intracellular delivery of phosphoinositides

Product Number: P-9C2R

Contents:

Catalog #	Description	Molecular Weight	Quantity
P-9C2R	Histone H1-TMR*	~26,730	2 x 10 nmoles

Storage:
This carrier is lyophilized. Protect from moisture and light and store at -20 ºC until reconstituted. Reconstitute with water or other aqueous solutions and store at 4ºC in the dark after reconstituting for up to 3 months. Multiple freeze thawing is not recommended. *Note: phosphate buffers are not recommended and may alter complex formation with phosphoinositides.* We do not recommend storing carriers and PIPs together as complexes.

Use:
Carriers are used to deliver phosphoinositide polyphosphates into living cells. This carrier has successfully delivered the following phosphoinositides into cells: PtdIns(4,5)P_2, PtdIns(3,4)P_2, PtdIns(3,4,5)P_3, and their fluorescent-derivatives.

References:
1. Ozaki, S., DeWald, D.B., Shope, J.C., Chen, J., Prestwich, G.D. Intracellular delivery of phosphoinositides and inositol phosphates using polyamine carriers. *Proc Natl Acad Sci U S A* **97**, 11286-91 (2000).
2. Wang, Y.J., Wang, J., Sun, H.Q., Martinez, M., Sun, Y.X., Macia, E., Kirchhausen, T., Albanesi, J.P., Roth, M.G., Yin, H.L. Phosphatidylinositol 4 phosphate regulates

targeting of clathrin adaptor AP-1 complexes to the Golgi. *Cell* **114**, 299-310 (2003).
3. Maffucci, T., Brancaccio, A., Piccolo, E., Stein, R.C., Falasca, M. Insulin induces phosphatidylinositol-3-phosphate formation through TC10 activation. *Embo J* **22**, 4178-89 (2003).
4. Larsen, M., Hoffman, M.P., Sakai, T., Neibaur, J.C., Mitchell, J.M., Yamada, K.M. Role of PI 3-kinase and PIP3 in submandibular gland branching morphogenesis. *Dev Biol* **255**, 178-91 (2003).
5. Weiner, O.D., Neilsen, P.O., Prestwich, G.D., Kirschner, M.W., Cantley, L.C., Bourne, H.R. A PtdInsP(3)- and Rho GTPase-mediated positive feedback loop regulates neutrophil polarity. *Nat Cell Biol* **4**, 509-13 (2002).

***TMR** = Tetramethylrhodamine (maximal excitation at 555 nm, maximal emission 580 nm)

Frequently-Asked Questions regarding the Echelon Shuttle PIP™ System

Echelon developed the Shuttle PIP™ system based on initial observations made in the laboratories of Drs. Glenn Prestwich and Daryll DeWald; and first published in the Proceedings of the National Academy of Sciences in 2000(1). Since then, several other research papers have been published further demonstrating the utility of this technology(2-6).

The terms "PIP" and "lipid" below refers to any one of seven possible phosphoinositides or phosphatidylinositol phosphate.

- **Echelon offers two kits: Shuttle PIP™ and Signal PIP™. Which one should I order?**

Shuttle PIP™ kits contain relatively more fluorescent PIP (any one of 8 Phsophatidylinositol phosphates), fluorescent shuttles, and are designed more for fluorescent microscopy experiments. Signal PIP™ kits have relatively more non-fluorescent PIPn and are designed for cell physiology experiments. Please refer to the catalog section of our web site for kit contents and current pricing (www.echelon-inc.com)

- **Will the Echelon Shuttle PIP system work with my cell-line (or primary cells, or organ culture system, etc)?**

We have not validated this system will all possible cell-lines and cell culture conditions, and you might be required to perform optimization experiments in your system. We have successfully delivered fluorescent phosphoinositides into NIH-3T3, 3T3-L1, Primary cardiac fibroblasts (Rat and Mouse), HeLa, MDCK, HL-60, BMMC (bone marrow derived mast cells), *A. thaliana* root-tip cells, *E. coli*, and the protist, *C. parvum*.

- **The kit comes with several carriers, which one should I use?**

We provide several carriers so that you can determine which carrier will be most effective in your system. If you desire to deliver bisphosphorylated PIPs or PIP3, we suggest trying Histone H1 (Carrier 2) first at concentrations less than 20 micromolar. If you are trying to deliver monophosphated PIPs, Carrier 3 is recommended.

- **How will the carriers affect my experiment?**

It is essential that you run a "carrier-only" control to determine how the carrier affects your system. Sometimes too much carrier can cause cell stress.

- **How much lipid and carrier do I need to add to my cells?**

The number of experiments you are able to perform with a kit depends on the concentrations used and the volume of medium covering your cells. We suggest minimizing the volume of your assay, starting with 1-20 micromolar PIP, then decreasing the amount of PIP to the minimum required to observe the desired response.

- **How long does it take for the lipid to gain entry into the cells?**

Depending on the cell type and assay conditions, significant entry is usually observed within 5-10 minutes. Often cells continue to brighten for 30 to 60 minutes.

- **Once inside cells, where does the lipid go?**

We have observed intracellular staining of many different patterns that depend on the phosphoinositide head-group and the length of the fatty-acyl chains. Also, the activation state of cells influences the cellular location of the fluorescent PIP analogs because they are potentially acted on by lipid kinases, phosphatases, and lipases.

- **What effect will the lipid have on my cells?**

There are now several examples published where delivery of phosphoinositides using this system activates physiological responses in the cell (calcium mobilization, cell motility, etc.). However, this is your experiment, and the answer to this question is in your hands and mind. Please contact Echelon (www.echelon-inc.com) with exciting results or any additional questions you might have.

REFERENCES

1. Ozaki, S., DeWald, D. B., Shope, J. C., Chen, J., and Prestwich, G. D. (2000) *Proc Natl Acad Sci U S A* **97**, 11286-11291.
2. Scheid, M. P., Huber, M., Damen, J. E., Hughes, M., Kang, V., Neilsen, P., Prestwich, G. D., Krystal, G., and Duronio, V. (2002) *J Biol Chem* **277**, 9027-9035.
3. Weiner, O. D., Neilsen, P. O., Prestwich, G. D., Kirschner, M. W., Cantley, L. C., and Bourne, H. R. (2002) *Nat Cell Biol* **4**, 509-513.
4. Larsen, M., Hoffman, M. P., Sakai, T., Neibaur, J. C., Mitchell, J. M., and Yamada, K. M. (2003) *Dev Biol* **255**, 178-191.
5. Maffucci, T., Brancaccio, A., Piccolo, E., Stein, R.C., Falasca, M. (2003) *Embo J* **22**, 4178-89.
6. Wang, Y.J., Wang, J., Sun, H.Q., Martinez, M., Sun, Y.X., Macia, E., Kirchhausen, T., Albanesi, J.P., Roth, M.G., and Yin, H.L. (2003) *Cell*, **114**, 299-310.

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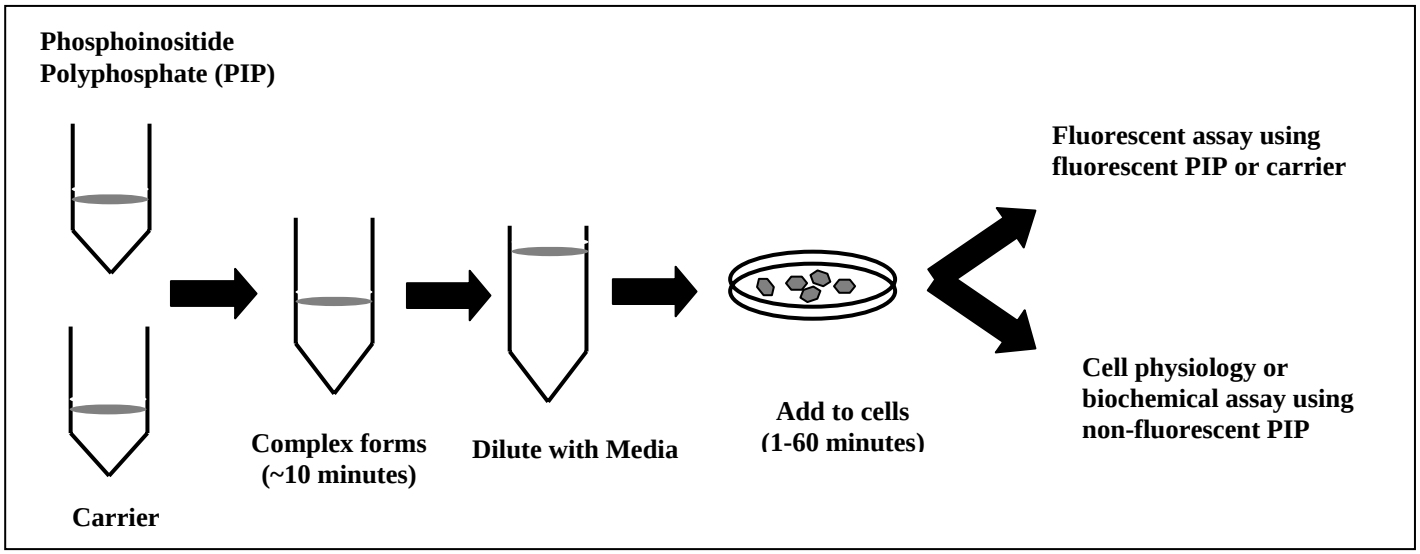
Shuttle PIP™ and Signal PIP™
Intracellular delivery of phosphoinositides

General Protocol

Add carriers at a 1 to 1 molar ratio with phosphoinositides (1:1) for 5-15 minutes in a test tube at room temperature. Brief vortex mixing and/or bath sonication may help complexes dissolve completely. Once formed, the complex is diluted to the desired final concentration in media or PBS containing cells (either adherent or suspended). *Note, we recommend final concentrations between 0.1 and 50 μM, but the optimal concentration for a given experimental system should be determined by each investigator.* Depending on the cell type, the phosphoinositide, and the system of study; significant uptake of fluorescent phosphoinositide or change in cell physiology is seen within 1 to 60 minutes. Slower-developing effects may require repeated application of carriers and phosphoinositides.



Product Test

Kits were tested using fluorescent phosphoinositides and carriers on NIH/3T3 cells imaged with a Nikon TS100 microscope. A successful test shows intracellular staining. Briefly, carriers and fluorescent phosphoinositides, 13 μL combined, were incubated in a test tube at a 1 to 1 molar ratio (~100 μM final concentration each) for 10 minutes at room temperature. The complex was diluted with 12 μL of medium and then added to 100 μL media covering adherent cells grown in an 8-well chamber slide on glass cover-slips. After 30-60 minutes, the dye-containing media was replaced with PBS and images were collected. The final shuttle and PIP concentrations on cells were both 10 μM. See Ozaki et.al. (2000) Intracellular delivery of phosphoinositides and inositol phosphates using polyamine carriers *Proc Natl Acad Sci* U S A **97** (21)11286-11291. Please visit our web site or contact our technical service department (1-866-588-0455) for additional questions.