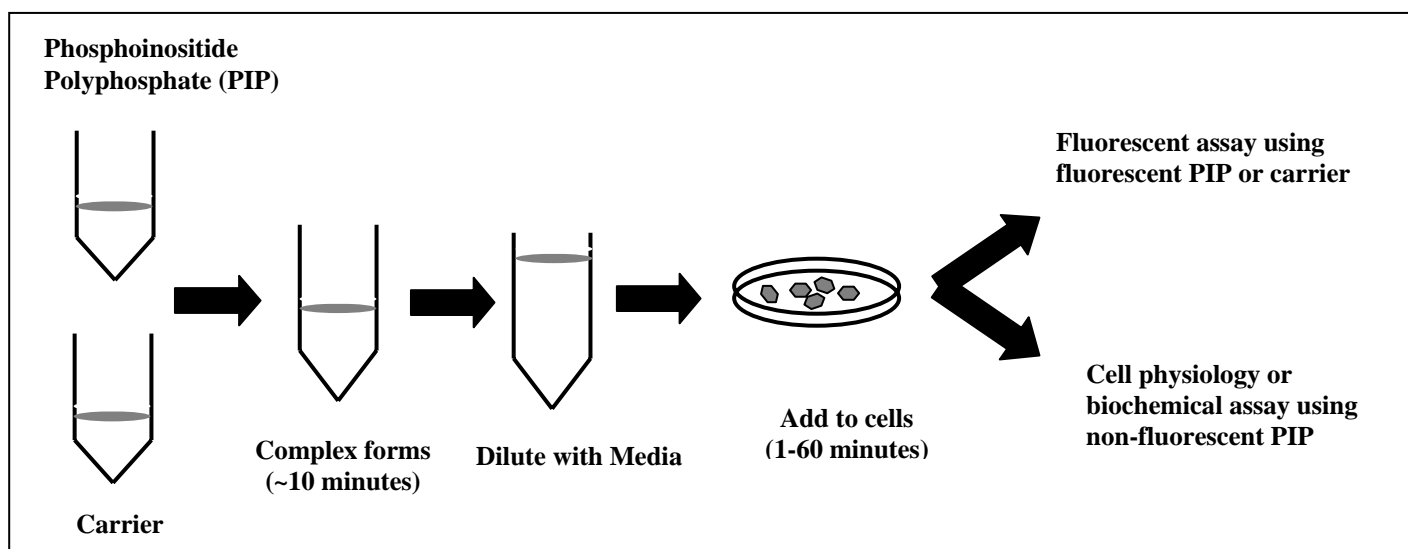


Shuttle PIP[™] and Signal PIP[™]

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 μ M, 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 μ L combined, were incubated in a test tube at a 1 to 1 molar ratio (~100 μ M final concentration each) for 10 minutes at room temperature. The complex was diluted with 12 μ L of medium and then added to 100 μ L 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 μ M. 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.