Echelon Biosciences Inc.

Shuttle PIP System – Frequently Asked Questions

Will the 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 50 micromolar. If you are trying to deliver monophosphorylated 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.

What buffers can I use?
Reconstitute PIPs and carriers in aqueous buffers or media for use. Vortex mixing, brief bath sonication and addition of small amounts of methanol, ethanol, or *tert*-butanol may facilitate complete dissolution of PIPs. Phosphate buffers are not recommended and may alter complex formation between carriers and PIPs. Visit www.echelon-inc.com for technical information for specific PIPs and carriers.

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 1-50 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 us at echelon@echelon-inc.com with exciting results or any additional questions you might have.

References

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