SPS, Modified 1/10/01 from Rosana Kapeller and Lucia Rameh

In Vivo Labeling of Cells with ^{32}P Inorganic Phosphate for Assaying PI3K Lipids

Notes: This protocol is designed for cells in 100 mm dishes. Typically these experiments are performed in the "radioactive room" and dishes are placed in large lucite boxes to minimize exposure to radioactivity. As of ~11/2000, all personnel who perform in vivo labelings must be approved by Harvard Radiation Safety on a person-by-person basis.

1. Incubate cells in PO_4-free media for 15 min. at 37 ^oC (5 ml).

2. Remove media. Add 5 ml of 200 μCi ^{32}P/ml in PO_4-free media for 4 hours.

Notes: It may be possible to do this in less time, but ideally one wants the labeling of ^{32}P to be "saturated" and incorporated into ATP in an equilibrium fashion. Larger (or smaller) amounts of radioactivity may also be used, if necessary.

3. Add the growth factor or stimulus to the cells in the presence of ^{32}P.

4. Wash cells one time with cold PBS or other appropriate media. Tilt the plate for a few seconds and remove the last drop of liquid.

5. Add 0.4 ml of 1 N HCl to the cells.

6. Add 0.4 ml of MeOH to dish. Scrape cells and place entire mixture in 1.5 ml eppendorf tubes. Use a clipped pipet tip to transfer, since the lysate will have a white precipitate, which is hard to pipet.

7. Add 0.4 ml $CHCl_3$ to tubes. Vortex, spin. Remove organic (lower) layer and set aside.

8. Extract organic phase (~400 ul) once with 400 ul of a mixture of MeOH: EDTA (0.1 M, pH 8.0) in a 1: 0.9 ratio.

9. Remove $CHCl_3$ (bottom) layer and place in eppendorf. Dry under Nitrogen stream.
Store this extract at -70 ^oC until deacylation.

A Few Obvious Rules:
1. Perform this experiment using a Mini monitor, lab coat, and radiation badge.
2. Cover work benches with paper to avoid contamination.
3. Place all liquid waste in "kitty litter".
4. Record all uses of radioactivity.
5. Monitor all work areas pre- and post-experiment. Monitor lab coat, hands, etc. Dispose of all dry materials in radiation waste.

TIPS: use good quality epp tube. Some brands don't seal well and will leak during vortexing. When vortexing radioactive organic solvents, use a kimwipe around the tube to avoid spraying radioactivity around. When pipeting organic solvents, saturate the pipet tip first, to avoid dripping. When transferring the chloroform from one tube to another, hold both tubes in one hand to avoid having to move the pipet tip through a long distance.