In Vivo PI Turnover Assay

Labeling Cells and Assaying

Buffers and Reagents Needed

Inositol-free DME
1M LiCl
[^{3}H]Inositol
Phosphate-buffered saline (PBS)
5% Trichloroacetic acid (TCA)

1. Grow cells to 50%-70% confluence in 6-well dishes. Remove medium and replace with DME: Inositol-free DME (1:1) containing 4% fetal calf serum to which has been added 0.75 uCi/ml [^{3}H]inositol.

2. Grow cells for two days in labeling media.

3. Before assay, add 10 mM LiCl to cultures and allow to incubate at 37° for 30 min.

4. Stimulate with growth factors for desired time (usually 15 min for single time point) Remove media. Wash cells with PBS.

5. Stop reaction by addition of 1 ml of 5% TCA.

Purification of IPs on Dowex Columns

Solutions needed:

1 M Formic acid stock
60 mm formic acid + 5 mM sodium tetraborate
1 M ammonium formate in 0.1 M formic acid

1. Scrape monolayers and transfer material to Eppendorf tubes. Wash plates with 0.5 ml water and add to the Eppendorf tubes.

2. Spin in microfuge for 5 min to sediment the TCA precipitate. Transfer the supernatants to 12 x 75 glass tubes. The pellets can be dissolved in 1 N NaOH and used for protein assay.

3. Add 1 ml diethyl ether and vortex 30 sec. Remove the upper ether layer.

4. Apply the samples to 3/4 ml Dowex 1-X8 columns that have been pre-wet with water.

5. Wash each column with 7 ml 60 mM formic acid, 5 mM sodium tetraborate

6. Elute with 5 ml of 0.1 M formic acid, 1.0 M ammonium formate

7. Add 15 ml Tru-Count scintillation fluid. Cap and shake. Count on program 10.

Note:
To separate the individual inositol phosphates, elute sequentially with 5 ml:

A) 0.2 M ammonium formate in 0.1 M formic acid for IP
B) 0.4 M ammonium formate in 0.1 M formic acid for IP_2
C) 1.0 M ammonium formate in 0.1 M formic acid for IP_3

Columns can be recycled by washing with ~10 ml 1.5 M ammonium formate in 0.1 M formic acid followed by 10 ml H_2O.