MEI Protocol

PLC assay

1. Plate 10^5 cells per well on 12 well plates.

2. A day after, at 60-70% cell confluence, transfect cells with 1 μg of plasmid DNA using Lipofectamine Reagent.

3. After 24 hr, wash cells with PBS. Add 0.4 ml of DMEM (with no inositol), 10% dialyzed FCS and 10 μCi/μl of myo-(2-^{3}H)inositol(Amersham): 100μl of label per 10 ml of medium. Incubate the cells for 20 h.

4. Clean the cells with PBS. Add 0.2 ml of DMEM (no inositol), 10% dialyzed FCS and 10 mM LiCl. Incubate at 37°C for 25min.

5. Stop the reaction with 0.2 ml of 10 mM cold formic acid. Wait 30min.

6. Prepare eppendorf tubes with 100 μl of neutralizing mixture:
   per well
   12 μl H2O
   8 μl PH indicator (Phydrion VWR)
   5 μl 0.5 M EDTA
   75 μl 2M KOH

7. Add 200 μl of the sample to the eppendorf tubes with the neutralizing solution. Vortex. Adjust the PH with 1M HCl or 2M KOH to a green color.

8. Centrifuge the sample 2 min at 4°C. Take all the supernatant to load the column.

9. Make the column by adding 500 μl(shaking the bottle) of H_2O/AG1-X8 anion exchange column(200-400 meshes, formate form, Bio-rad). Let all the liquid pour out of the column and add 1 ml of H_2O. Let the water pour out and add the sample.

10. After the sample has completely enter the column add 3.5 ml of 5 mM borax and 60 mM sodium formate. Repeat this step.

11. Elute the column with 1.5 ml of 1 M amonium formate and 0.1 M formic acid. Repeat. Mix by vortex. Take 1 ml to count with 10 ml of scintilation fluid.