

PI3-kinase assay

lipid kinase assay

PI3 Kinase Family

- Reaction solution (prepare as 3fold concentrated stock).
20 mM TrisHCl pH 7.4
4 mM MgCl₂
100 mM NaCl
- ATP mixture:
240 μM ATP, 1 μCi γ³²P-ATP/12.5 μl
- Lipid substrate vesicles:
800 μM phosphatidylinositol (Jena Bioscience) in water. Sonicate the emulsion 1 h in a water bath sonicator

Reaction mixture (add in the indicated order!):

20 μl water
25 μl reaction solution
12.5 μl lipid vesicles
5 μl sample (dilute as appropriate)

Incubate 5 min at room temperature and start reaction by addition of:
12.5 μl ATP mixture

Incubate 15 min at 37°C on a thermo-mixer

Stop reactions by addition of 150 μl 1 M HCl. Vortex.

Extract the lipids as follows: Add 450 μl of chloroform/methanol (1:1). Vortex, centrifuge to separate the phases and remove the upper (water) phase. Wash the organic layer twice with 200 μl 1 M HCl. Apply an adequate amount of the washed chloroform phase onto a silica gel TLC plate and run in 2 M acetic acid/isopropanol (1:2). Expose the plate for visualization and quantitation of phosphatidylinositol 3-phosphate spots. Alternatively, an aliquot of the washed chloroform phase may be counted by liquid scintillation.

Notes:

We use PI since this lipid is a lot cheaper than PI-4,5-P₂.