

PI 3-Kinase Assay Kit

Detection of PI 3-Kinase activity

Cat. No.	Amount
PR-943	8 x 12 assay points

For *in vitro* use only
Quality guaranteed for 12 months
Store at -20°C

Avoid freeze / thaw cycles

Kit contents

2x Reaction Buffer (2 x 1.5 ml)
ATP, 1 mM (400 μ l)
Lipid Substrate (8 x 100 nmol)
Control PI 3-Kinase (5 μ g)

To be provided by you

$[\gamma^{32}\text{P}]\text{-ATP}$
HCl, 1 M (300 μ l per assay point)
chloroform / methanol, 1:1 (300 μ l per assay point)
acetic acid / isopropanol, 1:2, 2 M
silica gel TLC plates
scintillation counter

Description

PI 3-Kinase Assay Kit provides a highly efficient and easy-to-handle tool for the sensitive detection and quantification of PI 3-Kinase activity or the screening for PI 3-Kinase inhibitors.

The activity of PI 3-Kinases is measured by the amount of radioactively labeled $[\gamma^{32}\text{P}]\text{-ATP}$ incorporated into lipid substrate. The substrate is thereafter separated using thin layer chromatography (TLC) and its radioactivity detected on a scintillation counter.

Background

Phosphoinositide 3-kinases (PI3Ks) are a family of enzymes that play a pivotal role in important cellular regulatory mechanisms. PI3Ks are capable of phosphorylating the 3'-OH position of phosphoinositide lipids (PIs) generating lipid second messengers. Their function has been linked to the regulation of numerous biological processes including cell growth, differentiation, survival, proliferation, migration. On the basis of structural similarities and substrate specificity, the PI3K family is divided into three classes termed I, II, and III.

All human class I members are heterodimers consisting of a catalytic subunit (MW approx. 110 kDa) and a non-catalytic subunit (MW 50, 55, 85, 87 or 101 kDa). They are known to phosphorylate phosphatidylinositol (PI), phosphatidylinositol-4-mono-phosphate (PIP) and phosphatidylinositol-4,5-bisphosphate (PIP2) *in vitro* but have a strong preference for PIP2 *in vivo*.

Preparation of the $[\gamma^{32}\text{P}]\text{-ATP}$ Mix

Prepare a mix of ATP and $[\gamma^{32}\text{P}]\text{-ATP}$ to obtain an activity of 1 μ Ci at a concentration of 240 μ M. 10 μ l $[\gamma^{32}\text{P}]\text{-ATP}$ Mix are required per assay point.

Preparation of Lipide Substrate Vesicles

Each Lipid Substrate Vial contains the amount of PI required for the preparation of 12 assay points. Fill up the Lipid Substrate Vial with 125 μ l water per vial. Sonicate the emulsion 1 h in a water bath sonicator.

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Kinase Assay

To prepare the Kinase Assay mix the following components for each assay point in separate vials.

Reaction Buffer: 25 μ l

Lipid Substrate Vesicle: 10 μ l

PI 3-Kinase Sample diluted as appropriate: 5 μ l

- Incubate 5 min at room temperature
- Add 10 μ l [γ^{32} P]-ATP Mix to start the reaction
- Incubate 15 min at 37°C
- Stop the reaction by addition of 100 μ l HCl (1 M) and vortexing

Extraction of the lipids

- Add 300 μ l of chloroform / methanol (1:1)
- Vortex the mix and centrifuge to separate the phases
- Remove the upper aqueous phase
- Wash the organic layer with 200 μ l HCl (1 M) and remove the water phase

Quantification

- Transfer 100 μ l of the organic phase onto a silica gel TLC plate and run it in 2 M acetic acid / isopropanol (1:2)
- Expose the plate for visualization and quantification of PI 3-Kinase spots in a scintillation counter
- Alternatively, an aliquot of the washed chloroform phase may be counted by liquid scintillation

Selected References:

- Ahn et al. (2004) PIKE (Phosphatidylinositol 3-Kinase Enhancer)-A GTPase Stimulates Akt Activity and Mediates Cellular Invasion. *J. Biol. Chem.* **279-16**:16441
- Dan et al. (2004) Phosphatidylinositol-3-OH kinase/AKT and survivin pathways as critical targets for geranylgeranyltransferase I inhibitor-induced apoptosis. *Oncogene* **23**:706
- Fruman et al. (1998) Phosphoinositide kinases. *Annu Rev Biochem.* **67**:481-507.