# Expression and Purification of PI3 Kinase alpha and Development of a Luminometric PI3 Kinase Activity Assay

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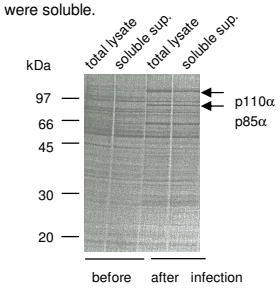
## Abstract

PI3 kinases (PI3Ks) are important targets for drug development as many steps in the PI3 kinase signaling pathway are mutated, hyperactivated or overexpressed in human cancers. Here, the human class Ia PI3 kinase p110alpha catalytic and the p85alpha regulatory domain were co-expressed in Sf9 cells, and purified. A luminometric ATP depletion assay to measure the activity of p110alpha/p85alpha was established. The assay was optimized by testing different lipids and lipid mixes as substrates using various kinase and lipid concentrations. Furthermore, the autophosphorylation status of PI3K alpha was checked and the IC50 value for the known PI3K inhibitor wortmannin was determined. The assay is homogenous, does not need any labeled substrate and is potentially suitable for high-throughput screening to identify PI3K inhibitors.

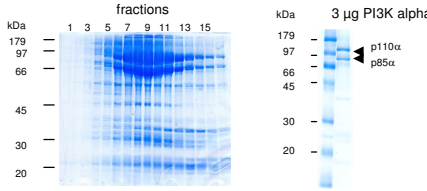
## Co-expression of PI3K p110alpha and p85alpha

Sf9 cells were infected with PI3K p110alpha and p85alpha recombinant Baculoviruses at an MOI of 2:1. An approximately 1:1 ratio of the two proteins were obtained in the crude extract. Both proteins were soluble.




## Purification of PI3K alpha

PI3K catalytic p110alpha subunit was expressed with a histidine-tag, whereas p85 regulatory subunit did not contain any tag. Purification was done using Ni-affinity chromatography. A single peak eluted containing both the catalytic and regulatory PI3K alpha subunits. The purity of the obtained PI3Kalpha was approximately 85 %.

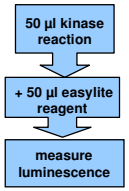



## Luminometric ATP depletion assay for PI3K

A luminometric ATP depletion assay (easylite from PerkinElmer), a well established protein kinase test, was optimized to measure PI3K activity. The assay is based on the production of light caused by the reaction of ATP with firefly luciferase and D-Luciferin.

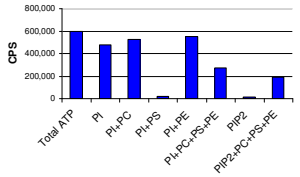
ATP + D-Luciferin + O2 $\xrightarrow{\text{Luciferase}}$ Oxyluciferin +Mg2+ + AMP + PPi + CO2 + light

The emitted light is proportional to the ATP concentration, which decreases during the kinase reaction.
The signal is read upon addition of a single reagent after completion of the kinase reaction.



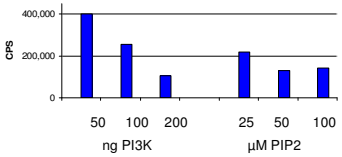

## Lipid substrates

Since different lipids and lipid mixtures have been described suitable to serve as PI3K substrates, some of them were tested. PIP2 seemed to be the best substrate.



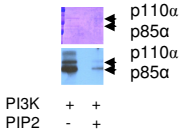

## Enzyme and substrate concentration

When testing PI3K alpha quantities between 50 and 200 ng per reaction, a decrease of signal was seen which correlated with the PI3K amount. When testing different PIP2 substrate concentrations, 50 µM seemed to be optimal, since 25 µM resulted in a lower decrease of signal intensity, whereas 100 µM gave the same results as 50 µM.



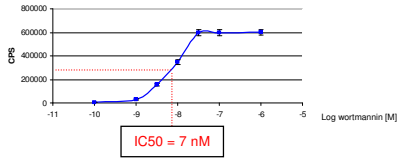

## Autophosphorylation of PI3K

PI3 kinase has been described to be autophosphorylated. Since ATP depletion assays cannot distinguish between ATP consumption because of autophosphorylation or substrate phosphorylation, the reaction was performed in the presence of radioactive ATP and autophosphorylation was analyzed by SDS-PAGE and autoradiography. In the presence of a substrate autophosphorylation is negligible.




## Inhibition of PI3K alpha by wortmannin

The well known PI3K inhibitor wortmannin was tested at increasing concentrations and the IC50 value was determined.




## Conclusion

The developed PI3 Kinase activity assay
- is a homogeneous assay
- does not require a labeled substrate
- is easy to perform
- does not need special instrumentation
- is potentially suitable for high-throughput screening

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