

Homogenous Kinase Assay using ATPlite™



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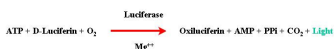
1 Abstract

ATPlite can be used to measure kinase activity in a homogeneous assay format without the need for labeled substrates, labeled enzymes or phosphospecific antibodies. ATPlite is an Adenosine Triphosphate monitoring system based on firefly (Photinus pyralis) luciferase. The assay system is based on the production of light caused by the reaction of ATP with added luciferase and D-luciferin. The emitted light is proportional to the ATP concentration. Kinase enzymes catalyze the transfer of a phosphate group from ATP to the substrate. ATPlite is used to measure the depletion of ATP, which is directly proportional to the amount of kinase activity. Since protein kinases are involved in a wide variety of cellular functions, their role in disease states has drawn considerable interest as drug discovery targets. This assay can significantly increase throughput and can be easily miniaturized and adapted to HTS robotic systems.

2 Introduction

Luminescence Assay Systems:

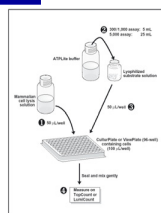
- A longer lasting and superior signal for a variety of cell-based and in vitro applications for high throughput and research formats.



Features and Benefits:

- High sensitivity
- Excellent linearity
- Simplicity
- Can be used for many kinase/substrate combinations
- Miniaturizable

3 ATPlite Assay System Adapted for Homogeneous Kinase Assays



ATPlite assay system for Homogeneous Kinase assays
– Instead of wells containing cells, add substrate and enzyme

Luminescence signal for ATPlite can be read on TopCount, Microbeta, Victor5V, EnVision, ViewLux or any other luminescence reader

4 Critical Materials

- ATPlite 300 assay kit (PerkinElmer Catalog # 6016943)
 - 1 X 20 mL of Cell Lysis Solution
 - 1 X 20 mL of Substrate Buffer Solution
 - 3 Vials of Substrate (Luciferase/Luciferin) Solution (Lyophilized)
 - 1 Vial of ATP Standard (Lyophilized)
 - Instruction Booklet
- Substrates
 - PKA Kemptide Substrate – Promega Catalog # V5601
 - Src Substrate Peptide – Upstate Catalog # 12-140
- Enzymes
 - PKA Enzyme – Promega Catalog # V5161
 - Src Enzyme – Upstate Catalog # 14-117
- OptiPlate™, 96, White – PerkinElmer Catalog # 6005290

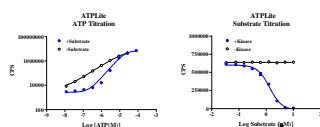
5 Methods

ATPlite Assay Protocol

- 10 µL Kinase Substrate or Buffer (for negative control)
- 10 µL ATP
- 10 µL Enzyme
- 20 µL Assay Buffer
- Incubate for 30 min at RT (PKA) or 4 Hours at 30°C (Src)
- 50 µL ATPlite Substrate solution
- Dark Adapt for 10 Minutes and Read the Luminescence

6 Results

ATPlite – PKA Assay

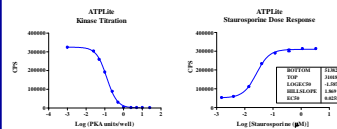


The optimal ATP concentration was determined by measuring the largest change in luminescence signal in wells with and without substrate.

The optimal substrate concentration was determined by measuring the amount of substrate that gives the least luminescence signal.

7 Results (Cont.)

ATPlite – PKA Assay

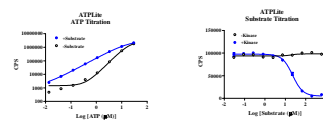


The optimal kinase concentration was determined by measuring the amount of kinase that gave luminescence signal in the linear part of the curve.

A dose response curve with stauroporin, a kinase inhibitor was performed with the following conditions: 1 µM ATP, 5 µM substrate, and 0.5 units/well kinase.

8 Results

ATPlite – Src Assay

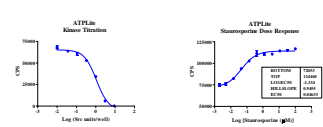


The optimal ATP concentration was determined by measuring the largest change in luminescence signal in wells with and without substrate.

The optimal substrate concentration was determined by measuring the amount of substrate that gives the least luminescence signal.

9 Results (Cont.)

ATPlite – Src Assay



The optimal kinase concentration was determined by measuring the amount of kinase that gave luminescence signal in the linear part of the curve.

A dose response curve with stauroporin, a kinase inhibitor was performed with the following conditions: 0.5 µM ATP, 200 µM substrate, and 1 unit/well kinase.

10 Conclusions

- ATPlite can be used for homogeneous kinase assays without the need for substrate or antibody labeling
- Phosphorylation by a variety of substrate/kinase combinations can be monitored
- Assays can be miniaturized for high throughput screening
- Assays can be easily adapted to high throughput screening robotic systems
- Due to the simplicity of the ATPlite system, assays can be rapidly developed

Coming Soon!
EasyLite-Kinase