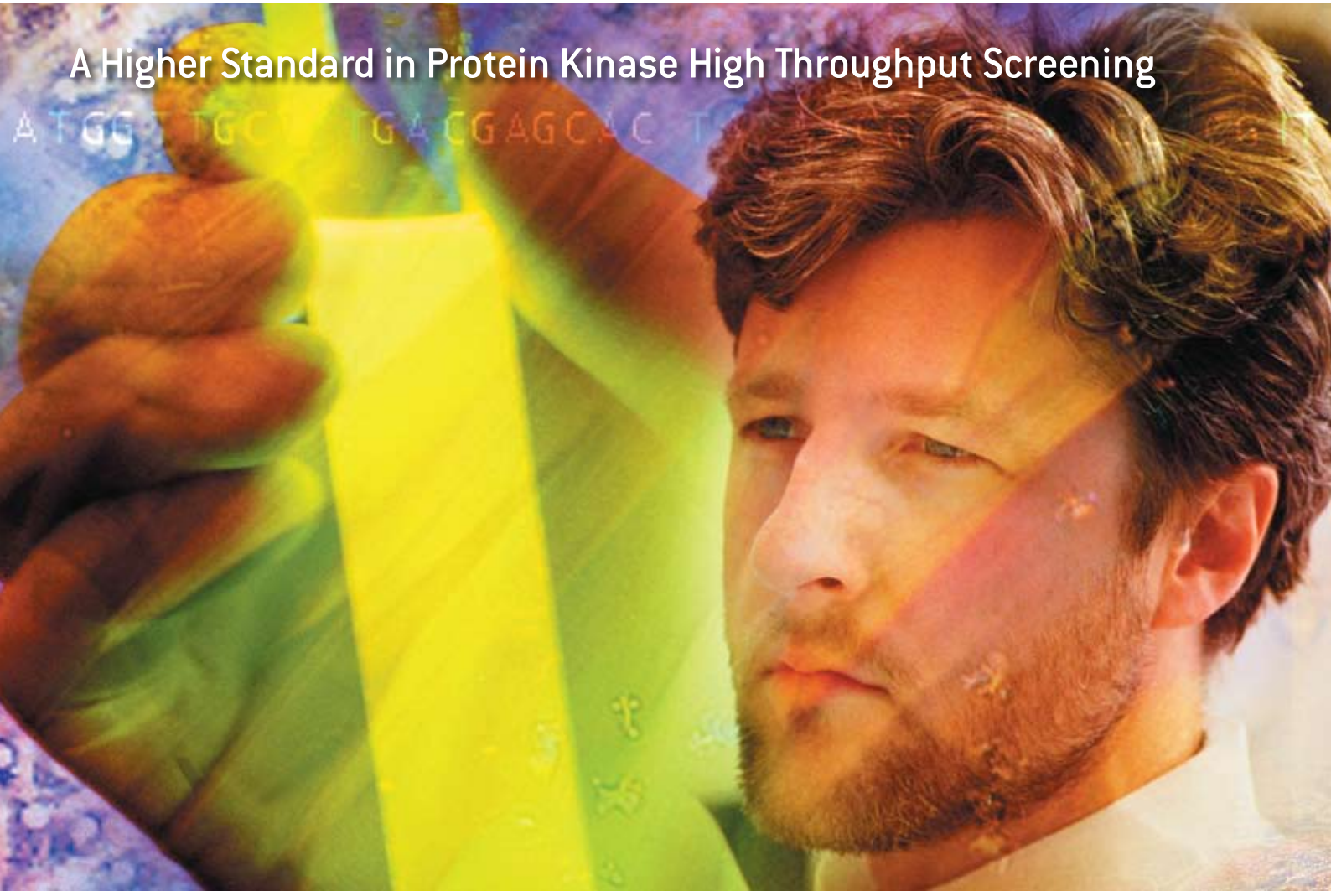
Lonza

PKLight® HTS Protein Kinase Assay Kit



Studies into protein kinases have been ongoing since the late 1960s, and in excess of 500 different kinases have been identified. Their importance in the signal transduction pathways of many diseases was soon realized, and they have been investigated as potential drug targets for over 25 years, making them critical screening targets.

The first steps to understanding the mechanism of bioluminescence occurred in 1947.[1] The period that followed was marked by a rapid series of reports describing the central role of ATP in firefly bioluminescence. Since then, ATP measurement by the luciferase-luciferin reaction for cell viability, proliferation and cytotoxicity have become commonplace. This was aided by the development of the first commercial ATP detection kits by Lonza, namely ViaLight®, ToxiLight® and ApoGlow® BioAssays in the mid 1990s.

At Lonza, we combined our expertise in bioluminescence with the need for a rapid protein kinase assay kit, and developed the patented technology utilized by the PKLight® HTS Protein Kinase Assay Kit. This has proved an essential tool in research and drug discovery development efforts in diseases such as cancer, arthritis and diabetes.[2]

SP006125 Inhibition of JNK2α2

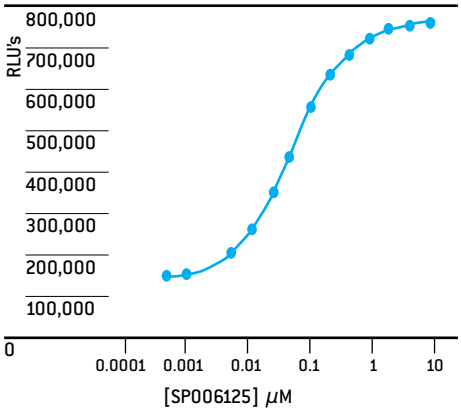


FIGURE 1. SP006125 inhibition of JNK2α2, 0.005 - 5 μM. 50 ng/well JNK2α2, 10 μg/well c-Jun (substrate) and 2 μM ATP in a total assay volume of 40 μl was incubated for 1 hour at room temperature. An IC_{50} value of 0.04 μM SP006125 was determined using the PKLight® Assay.

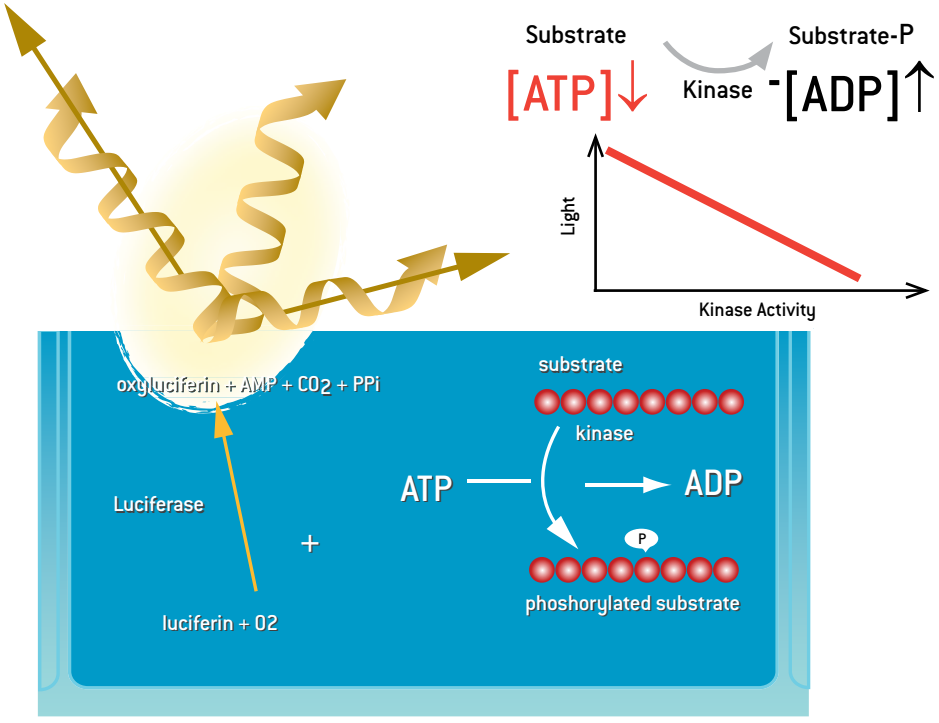
The PKLight® Assay Kit is a homogeneous ATP consumption assay and has been designed specifically for use in the high throughput screening of kinase activity. This patented bioluminescent detection method provides a generic endpoint determination of virtually all protein kinase assay kits. The system delivers Z´ values of >0.7 with minimal interference for good clean hits. [3]

How The Assay Works

The PKLight® Assay Kit uses luciferase bioluminescence to measure ATP consumption as a result of kinase phosphorylation of the target substrate. Cleavage of the γ–phosphate from ATP associated with the protein kinase activity results in a decrease in the ATP concentration, which is measured by bioluminescent detection of the remaining ATP (See Figure 2).

The assay can easily be optimized for each kinase/substrate pair. Reproducible data makes the assay ideal for IC_{50} determination of screen compounds with signal stability suitable for HTS.

FIGURE 2



Advantages of the PKLight® Assay Kit

- **Sensitivity:** A small change in ATP concentration leads to a large change in signal; this high resolution enables the identification of low potency inhibitors (See Figure 3)
- **Label Free:** Allows for rapid development, is cost effective and miniaturizable to 384 and 1536-well platforms and beyond
- **Clean Hits:** Low false positive rate and few artifacts allows for a high confirmation rate and a high level of confidence in your results
- **Peptide/Protein Substrates:** The assay measures ATP concentration so it is independent of substrate type; the substrate can be a peptide or even another kinase

The PKLight® Assay Kit contains all of the detection reagents necessary for the *in vitro* assay of protein kinase activity. The assay platform may be used in a variety of ways to suit the requirements of the kinase/substrate pair and the particular demands of the screening group in 96, 384, or 1536-well microtiter plate formats. The reagents can be supplied in custom formats to suit a particular screening need.

Easy, Homogeneous Assay

The PKLight® Assay Kit is very simple and easy to use. All you need in addition to the assay is the kinase of interest, the substrate and ATP:

1. Incubate kinase and substrate with test compound in the presence of ATP
2. Stop the reaction using the Stop Solution (at this point the ATP concentration in the assay mixture will be fixed and will reflect the kinase activity during the incubation period)
3. Add ATP Detection Reagent and measure the light emission

ATP Standard Curve 100 pM - 20 μM

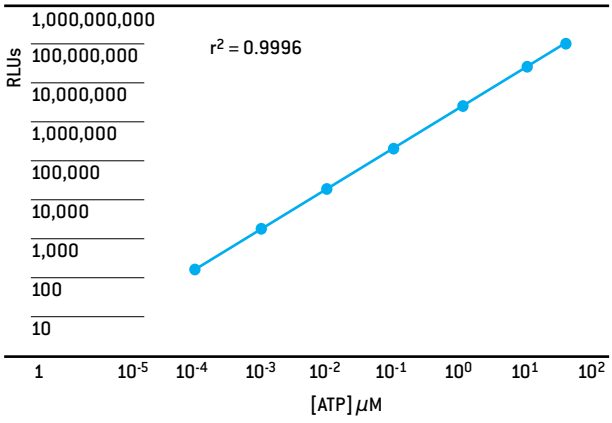
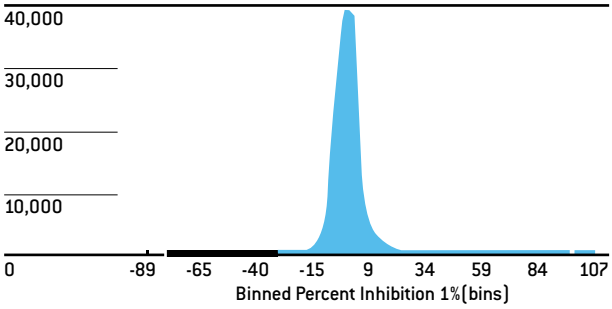


FIGURE 3. Correlation of ATP concentration and light output for the PKLight® Assay. The PKLight® Assay has an exceptional assay window and is linear to 20 mM ATP. A small reduction in ATP with the PKLight® Assay will lead to a large change in signal. This resolving capacity allows for the detection of low potency inhibitors.

A Superior Method

Unlike screening technologies such as Homogeneous Time Resolved Fluorescence (HTRF), and the Scintillation Proximity Assay (SPA), the PKLight® Assay Kit does not require the use of radioactive beads, radiolabeled ATP, specially designed antibodies or specifically modified substrate sequences. You do not have to obtain new antibodies and beads for each substrate pair, saving time and money. Unlike SPA, the bioluminescent assay works with large molecules and is not affected by the size of the molecules in the reaction.

Inhibitor screen 400,000+ compounds in a 384-well format



Screening Set	Diverse Set
Plate Format	384
[Compound] μM	10.0
Number of compounds	444,321
Average % inhibition	0.7
Limit for positives	18%
Number of positives	3998 (0.9%)
Average Z´ factor	0.74 (±0.08)

FIGURE 4. An inhibitor screen of 400,000+ compounds using the PKLight® Assay in a 384-well format. Using a cut off of >18% inhibition, 3,998 compounds were deemed positive inhibitors of the kinase with an average Z´ value of 0.74. Data courtesy of Aimo Kannt, Assay Technologies/HTS, Aventis, Frankfurt

Example Kinases Tested

- JNK with cjun and ATF-2
- ERK with MBP
- MEK with MBP
- SAPK with MBP
- Raf-1 with inactive MEK-1
- PKA with Kemptide
- SRC with SRC peptide substrate

This list is not extensive. A number of serine/threonine and lipid kinases have been tested successfully in 96, 384 and 1536-well formats in external collaborations.

Ordering Information

PKLight® HTS Protein Kinase Assay Kit

Cat. No. LT07-500	500 tests
Cat. No. LT07-501	5,000 tests

For more information about the PKLight® HTS Protein Kinase Assay Kit, or for bulk reagent pricing, please contact your Lonza HTS Sales Specialist.

1. McElroy W.D. (1947) The energy source for bioluminescence in an isolated system *PNAS*: **Vol # 331 (II):** 342-345
2. Cohen P. (2000) The regulation of protein function by multisite phosphorylation. *Trends Biochem Sci.* **Vol 25:** 596-601.
3. Singh, P., Harden, B.J., Lillywhite, B.J., Broad, P.M. (2004) Identification of kinase inhibitors by an ATP depletion method. *Assay and Drug Development Technologies.* **Vol # 2(2):** 161-169.

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