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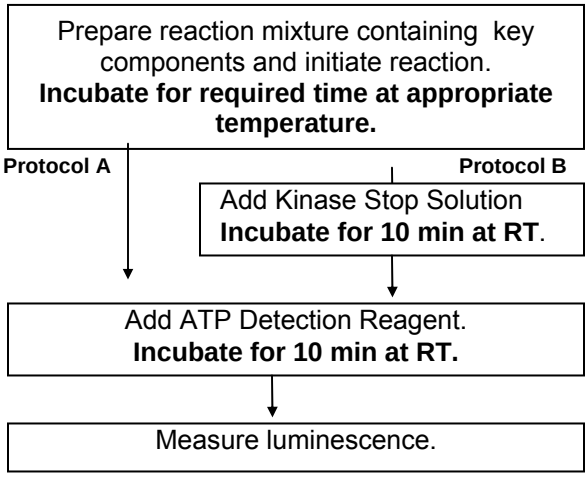
PKLight® Assay Kit

HTS Protein Kinase Assay

Instructions For Use

PKLight Assay Procedure
(For detailed assay procedure see relevant protocol)



Kit Contents

LT07-500 (Sufficient for a minimum of 500 assay points)

1. LT27-200 ATP Detection Reagent (ATP-DR). Lyophilized 1 x 10 ml bottle.
2. LT27-202 ATP-DR Reconstitution Buffer A. 1 x 10 ml bottle.
3. LT27-207 ATP-DR Reconstitution Buffer B (for use with Stop Solution). 1 x 10 ml bottle.
4. LT27-228 Kinase Stop Solution. 1 x 10 ml bottle.

LT07-501 (Sufficient for a minimum of 5000 assay points)

1. LT27-233 ATP Detection Reagent (ATP-DR). Lyophilized 2 x 50 ml bottle.
2. LT27-205 ATP-DR Reconstitution Buffer A. 1 x 100 ml bottle.
3. LT27-210 ATP-DR Reconstitution Buffer B (for use with Stop Solution). 1 x 100 ml bottle.
4. LT27-231 Kinase Stop Solution. 1 x 100 ml bottle.

The kit components should be stored at 2˚C-8˚C. Do not freeze. See kit label for expiry date of the whole kit. See bottle labels for expiry dates of individual components.

Please Note: PKLight® Reagents are available in bulk quantities based upon individual requirements. Your Lonza representative can advise you on the most suitable alternatives.

Intended Use

The PKLight Assay Kit is a generic, homogeneous assay designed for use in High Throughput Screening (HTS) of kinase activity using 96, 384, or 1536-well microtitre plates. The kit contains all reagents necessary for the assay and has been designed for use in a cell free system. All that is required from the user is a purified form of the kinase, a suitable substrate, buffer, and ATP. Extensive research has shown the assay is suitable for serine / threonine, tyrosine and lipid kinases. PKLight® Assay can be easily optimized for each kinase / substrate pair to rapidly produce data suitable for IC_{50} determination of screen compounds. This technology does not require radioactive beads, radiolabelled ATP or specifically modified substrate sequences.

Principles

PKLight® Assay exploits kinases' intrinsic ATPase activity, resulting in the cleavage of the γ-phosphate from ATP and its subsequent insertion into the target substrate. This results in the phosphorylation of the substrate and the conversion of ATP to ADP. PKLight® Assay measures the consumption of ATP and is based upon the bioluminescent measurement of the remaining ATP present in the wells after kinase activity.

$$\text{Substrate + ATP} \xrightarrow{\text{kinase}} \text{(Substrate – P) + ADP}$$

The assay utilizes the enzyme luciferase, which generates light from ATP and luciferin. By adding PKLight® ATP Detection Reagent to each of the completed kinase reactions, the consumption of ATP due to kinase activity can be accurately measured.

$$\text{ATP + Luciferin + O2} \xrightarrow[\text{Mg ++}]{\text{Luciferase}} \text{Oxyluciferin + AMP + } PP_i + CO_2 + \text{LIGHT}$$

The emitted light intensity is linearly related to the ATP concentration and is measured using a luminometer or beta counter. The assay is conducted at ambient temperature (18˚C-22˚C), the optimal temperature for the luciferase enzyme. Bioluminescence is now the most widely used method for the assay of ATP due to its very high sensitivity, wide dynamic range, and ease of use.

The sensitivity of PKLight® Assay is limited by the amount of ATP consumed during the kinase activity. It is important to optimize the kinase reaction to consume enough ATP. This can be done by adjusting the concentration of kinase, substrate, and/or ATP in the wells, and by extending the incubation time of the kinase reaction. The ATP detection assay performs optimally at pH 6.0 - 8.5 using ATP concentrations between 0.1 µM and 20 µM. (Figure 1).

The Kinase Stop Solution is designed for HTS systems. The kinase reaction can be run for a specific time and halted. The addition of the Kinase Stop Solution enables plates to be batch processed.

In conjunction with this, PKlight Assay offers a stable signal output with a half-life greater than 2 hours. Kinase activity is inversely proportional to the bioluminescent signal. (Figure 2).

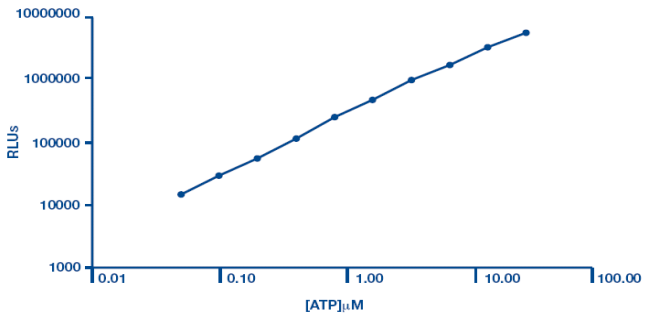


Figure 1: Bioluminescent Signal Output. Relative Light Units (RLUs) correlate proportionally with ATP concentration. The assay is linear over the range 0 - 20 µM ATP. (R^2 = 0.997).

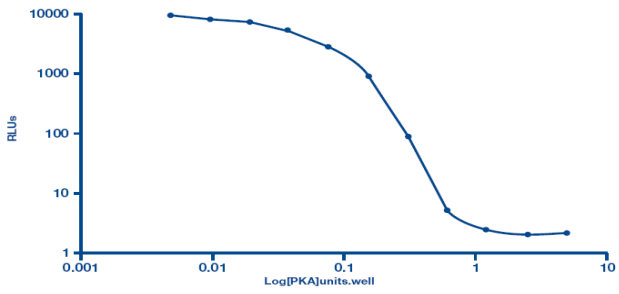


Figure 2: Bioluminescent signal of PKLight Assay is inversely proportional to kinase activity. Increasing concentrations of cyclic AMP dependent protein kinase (PKA) were incubated in kinase reaction buffer containing 40 mM Tris-HCl pH7.5, 20 mM $MgCl_2$, 0.1% (w/v) BSA, 5 µM Kemptide and 1 µM ATP.

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Outline of the Method

- The kit contains all the required reagents for the assay.
- For additional equipment required to perform the assay please see the Equipment section (p.3).
- Prepare kinase reaction mixture (reaction buffer containing reaction components) and initiate reaction. Recommended volume 40 µl.
- Incubate for required time and temperature.
- **Add Kinase Stop Solution - Optional.**
- Add ATP Detection Reagent (ATP-DR) .
- Incubate for at least 10 minutes at RT.
- Read luminescence.

Reagent Reconstitution and Storage

Please read this section carefully to ensure optimal performance for your assay. This procedure requires at least 15 minutes equilibration time.

The ATP Detection Reagent (ATP-DR) is supplied lyophilized and needs to be reconstituted in ATP-DR Reconstitution Buffer (supplied) to produce the working solution for use in the assay.

IMPORTANT: The kit is supplied with two different ATP-DR Reconstitution Buffers, A & B. If you do not want to use the Kinase Stop Solution, then ATP-DR Reconstitution Buffer A must be used. When working with the Kinase Stop Solution use ATP-DR Reconstitution Buffer B

1. 10 ml ATP Detection Reagent (ATP-DR)

- Add 10 ml of the appropriate ATP-DR Reconstitution Buffer into the vial containing the lyophilized ATP-DR.
- Replace the screw cap and mix gently.
- Allow the reagent to equilibrate for 15 minutes at room temperature.

2. 100 ml ATP Detection Reagent (ATP-DR)

- Add 100 ml of the appropriate ATP-DR Reconstitution Buffer into the vial containing the lyophilized ATP-DR.
- Replace the screw cap and mix gently.
- Allow the reagent to equilibrate for 15 minutes at room temperature.

Use reconstituted ATP Detection Reagent within 6 hours. Unused reagent can be aliquotted into polypropylene tubes and stored at –20°C or below for up to one month protected from light. Allow any frozen reagent to equilibrate to room temperature without the aid of artificial heat. Once thawed, reagent must not be refrozen.

3. ATP-DR Reconstitution Buffer A
This is provided ready for use. Store at 2˚C-8˚C when not in use.

4. ATP-DR Reconstitution Buffer B. For use with Kinase Stop Solution Only.
This is provided ready for use. Store at 2˚C-8˚C when not in use.

5. Kinase Stop Solution
This is provided ready for use. Store at 2˚C-8˚C when not in use.

Equipment

1. Materials and Instrumentation. (To be supplied by the user).

- **kinase (suitably pure and free from ATPase contaminants)**
- **kinase substrate**
- **ATP**
- **kinase reaction buffer**

PKLight® Assay requires a luminometer or beta counter. The parameters of the luminometer should be assessed and the conditions below used to produce the correct programming of the machine. If the luminometer has temperature control this should be set to 22˚C, the optimal temperature for luciferase.

Microplate Luminometers:
- Read time 0.1 second (integrated).

Beta Counters:
- Mode – out of coincidence or luminescence.
- Read time 0.1 second (integrated).

NOTE: The integrated read time of 0.1 second as suggested above is highly recommended. Integral read times can be adjusted but should be kept between 0.1 to 1 second.

2. Additional Equipment and Consumables.

a. 10 ml sterile pipettes
b. Opaque white microtitre plates suitable for luminescence measurements. The same microplates should be used with beta counters.
c. Multichannel micropipettes - 5-50 µl or a suitable micro-dispensing system

Protocol
The following protocols are suitable for both 96 and 384 well plate formats. If 1536 well plates are to be used then please contact Technical Service at biotechserv@Lonza.com.

NOTE: The outlined method of screening compounds for kinase activity using PKLight Assay is offered as a guideline. The volumes given can be adjusted relatively. The ratio of volumes of the kinase reaction mixture to Kinase Stop Solution (if required) and ATP Detection Reagent should remain constant.

Before performing the assay, the ATP Detection Reagent must be left for at least 15 minutes at room temperature to ensure complete rehydration.

Protocol A. (without Kinase Stop Solution)

1. Bring all reagents up to room temperature before use.
2. Reconstitute the ATP Detection Reagent (ATP-DR) in Reconstitution Buffer A (see p.2).
3. Prepare kinase reaction mixture and initiate reaction to give a final volume of 40 µl in each well.
4. Incubate for required time and temperature.
5. Program the luminometer to take a 0.1 second integrated reading.
6. Add 20 µl of reconstituted ATP-DR to the wells and incubate for 10 minutes at room temperature.
7. Read plate.

Protocol B. (With Kinase Stop Solution)

1. Bring all reagents up to room temperature before use.
2. Reconstitute the ATP Detection Reagent (ATP-DR) in Reconstitution Buffer B (see p.2).
3. Prepare kinase reaction mixture and initiate reaction to give a final volume of 40 µl in each well.
4. Incubate for required time and temperature.
5. Program the luminometer to take a 0.1 second integrated reading.
6. Add 10 µl of the Kinase Stop Solution to the reaction to stop further kinase activity.
7. Incubate at RT for 10 minutes.
8. Add 20 µl of reconstituted ATP-DR to the wells and incubate for 10 minutes at room temperature.
9. Read plate.

Interpretation of Results
PKLight Assay offers an easily established end point for the detection of kinases. The bioluminescent signal is inversely proportional to the activity of the kinase. Kinase activity will result in a reduced signal compared with controls. An inhibiting compound will reduce the consumption of ATP by the kinase and the signal will be higher than the inhibitor-free controls. PKLight Assay is a measure of the remaining ATP present after kinase activity. (See Figure 3).

In most cases the direct output from the luminometer (commonly Relative Light Units or RLUs) or beta counter (cpm) may be used to assess the enzymatic response to the target compound. This reaction is linear between 0 µM – 20 µM of ATP.

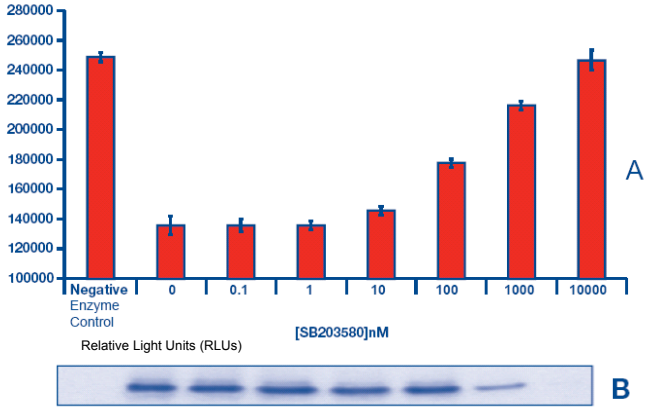

Figure 3: **A.** SB203580 inhibition of SAPK2ß phosphorylation of dephosphorylated Myelin Basic Protein SB203580 in varying concentrations was used to inhibit the phosphorylation of Myelin Basic Protein (MBP) by SAPK2ß. In comparison to the negative enzyme control, a concentration dependent effect was observed. At 10,000 nM SB203580, 100% inhibition was obtained. **B.** A western blot using anti phospho MBP was used to confirm kinase activity. 100% inhibition of SAPK2ß was seen at 10,000 nM correlating with the bioluminescent assay.

Troubleshooting

Interference and poor signal output

PKLight® Assay is designed to be resistant to DMSO up to a final concentration of 20% (v/v) and many other interfering compounds. Intensely red colored compounds will quench light emission and should be taken into consideration. The luciferase reaction requires Mg++ as a co-factor. Therefore, chelating agents such as EDTA should be avoided in high concentrations.

PKLight Assay can be used to identify compounds that may interfere with the assay by screening without the kinase present. A reduction in light output signal will be observed, if the test compound interferes with the PKLight Detection Reagent.

PKLight Assay runs optimally at pH 6.0-8.5 and at room temperature.

If the kinase reaction has been optimized for other assay systems with different sensitivities, the relative concentrations of each key component may need to be adjusted to allow for optimal performance with PKLight Assay.

Reaction volumes

These can be adjusted relatively to suit the format of the assay, i.e., 96 or 384-well and low volume plates. The assay has been optimized for the ratio of stop solution and detection reagent used to the kinase reaction volume.

If technical support is required please contact biotechserv@Lonza.com

Ordering Information

LT07-500 500 Tests
LT07-501 5000 Tests

For Research Use Only. Not for Diagnostic Use.

PKLight is a Protein Kinase Assay. PKLight and the method of detecting kinase activity and/or inhibition by the bioluminescent measure of ATP consumption are protected by UK patent GB 2,375,171 B; US patents 6,599,711 and 6,911,319, Swiss patent CH 694 096 A5, and other patents pending.

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