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Document # Inst-LT07-610 07/11

PPiLight™ inorganic pyrophosphate assay

Instructions for use

Safety

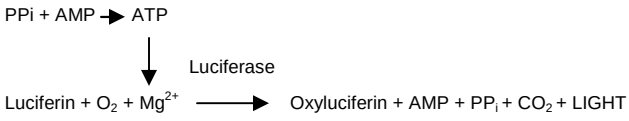
THESE PRODUCTS ARE FOR RESEARCH USE ONLY. Not approved for human or veterinary use, for application to humans or animals, or for use in clinical or *in vitro* procedures.

Description

Inorganic pyrophosphate (sometimes referred to as diphosphate, pyrophosphoric acid or PPi) is a small diphosphate molecule that is required as a substrate for or is the product formed from a number of different enzymatic reactions. Enzymes that utilize PPi as a substrate may include phosphotransferases (EC 2.7) and pyrophosphatases (EC 3.6.1.1). Enzymes that generate PPi are more numerous and may include cyclases (EC 4.6.1), hydrolases (EC 3) and ligases
(EC 6).

PPiLight™ assay is a non-radioactive bioluminescent assay for the detection of inorganic pyrophosphate. In the presence of PPi the detection reagent catalyses the conversion of AMP to ATP. The assay uses luciferase, which produces light from the newly formed ATP and luciferin following the reaction schematic shown in Figure 1.

Figure 1: Bioluminescent reaction



The amount of light produced is directly proportional to the amount of PPi present in the sample (Figure 2).

The assay does not require any radioactive substrates, beads, modified substrates or antibodies. It can be performed in 96-, 384- or 1536-well plates and is suitable for miniaturization.

PPiLight™ assay has linearity between 0.02 and 10 µM PPi with excellent Z' values. The PPi signal increases over time at a steady and constant rate proportional to the PPi concentration (Figure 3A). The linearity of the signal remains constant throughout the 1 hour incubation period (Figure 3B).

Figure 2:

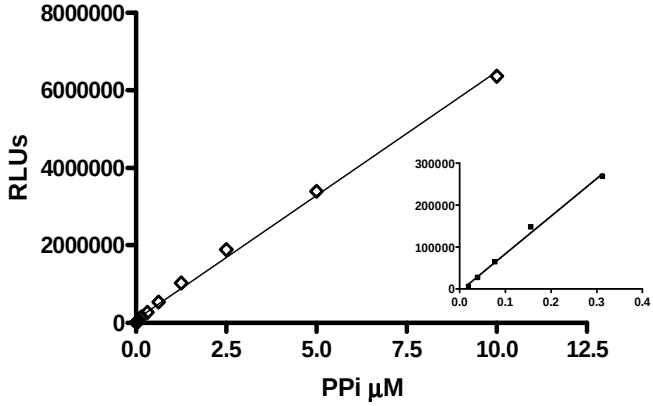


Figure 2: Typical linear PPi standard curve using the PPiLight™ assay Reagents (r^2 values > 0.99). The sensitivity for PPi is typically 0.02 µM with a linear range up to 10 µM in a 100 µl sample.

Figure 3A:

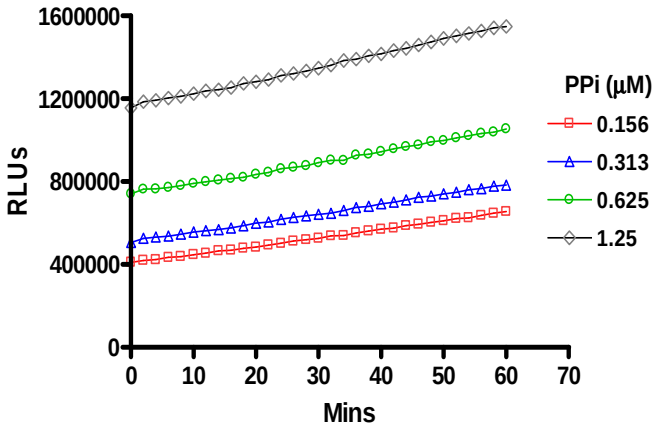


Figure 3A: The signal generated from varying concentrations of PPi was monitored over 1h and showed an increase. This was shown to be steady and constant proportional to the PPi concentration

Figure 3B:

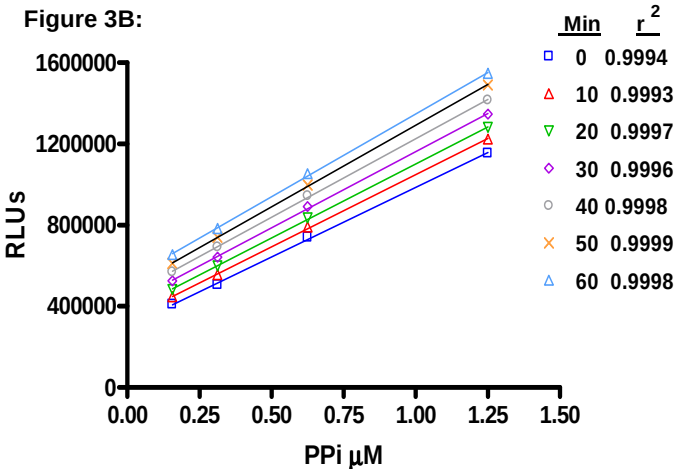
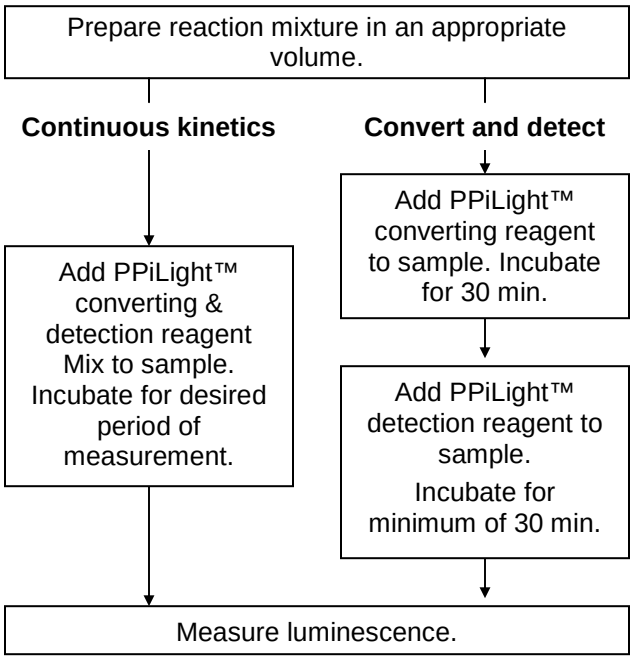


Figure 3B: The linearity of the signal generated with varying concentrations of PPi was assessed over 1h.

Protocol selection

Figure 4: Flow chart of PPiLight™ assay protocols



Kit contents

PPiLight™ inorganic pyrophosphate assay	**Cat No:** LT07-610	**Size** 500 tests

Component	**Cat No:**	**Size**
PPiLight™ converting reagent	LT27-263	10ml
PPiLight™ detection reagent	LT27-260	10ml
PPiLight™ reconstitution buffer	LT27-266	20ml

Kit components should be stored at 2℃-8℃. See kit label for expiration date of the whole kit. See bottle labels for expiration dates of individual components.

NOTE: PPiLight™ reagents are available in bulk quantities based on individual requirements. Your Lonza representative can advise you on the most suitable options.

Equipment and reagents

The PPiLight™ kit requires the use of 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

Microplate luminometer:

Read time: 0.1second (integrated)

Beta counters:

Mode: out of coincidence or luminescence

Read time: 0.1second (integrated)

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

Additional equipment and consumables

1. 10 ml sterile pipettes.
2. Opaque white wall microtiter plates suitable for luminescence measurements. The same microplates should be used with beta counters.
3. Multichannel micropipettes – 5 µl-50 µl or a suitable micro-dispensing system.

Reagent reconstitution and storage

See Figure 4 for selection of the appropriate protocol. Please read this section carefully to ensure optimal performance for your assay. This procedure requires at least 15 minutes equilibration time.

Convert and detect protocol

PPiLight™ converting reagent (lyophilized)

1. Add 10 ml of the reconstitution buffer to the vial containing the lyophilized PPiLight™ converting reagent.
2. Replace the screw cap and mix gently. Do not vortex.
3. Allow the reagent to equilibrate for 15 minutes at room temperature.

Use reconstituted PPiLight™ converting reagent within 6 hours or 24 hours if stored at 4℃.

Prolonged storage

Unused reagent can be aliquoted into polypropylene tubes and stored at -20℃ or below for up to 2 months protected from light. Allow to equilibrate to room temperature without the aid of artificial heat before use. Once thawed reagent must not be refrozen.

PPiLight™ detection reagent (lyophilized)

1. Add 10 ml of the reconstitution buffer to the vial containing the lyophilized PPiLight™ detection reagent.
2. Replace the screw cap and mix gently. Do not vortex.
3. Allow the reagent to equilibrate for 15 minutes at room temperature.

Use reconstituted PPiLight™ detection reagent within 6 hours or 24 hours if stored at 4℃.

Prolonged storage

Unused reagent can be aliquoted into polypropylene tubes and stored at -20℃ or below for up to 1 mont h protected from light. Allow to equilibrate to room temperature without the aid of artificial heat before use. Once thawed reagent must not be refrozen.

Continuous kinetics protocol

PPiLight™ converting reagent (lyophilized)

1. Add 10 ml of the reconstitution buffer to the vial containing the lyophilized PPiLight™ converting reagent.
2. Replace the screw cap and mix gently. Do not vortex.
3. Add the converting reagent to the vial containing the lyophilized PPiLight™ detection reagent.
4. Allow the reagent to equilibrate for 15 minutes at room temperature.

Use reconstituted PPiLight™ combined reagent within 6 hours or 24 hours if stored at 4℃.

Prolonged storage

Unused reagent can be aliquoted into polypropylene tubes and stored at -20°C or below for up to 1 month protected from light. Allow to equilibrate to room temperature without the aid of artificial heat before use. Once thawed reagent must not be refrozen.

Reconstitution buffer

This is provided ready for use. Store at 2ºC-8ºC when not in use.

Protocols

NOTE: 96- and 384- well plate formats only – for information on use with 1536 well plate formats contact Scientific Support.

Please ensure you have read the appropriate reconstitution instructions before starting. All reactions should be conducted in a final volume of 40 µl prior to assay.

See Figure 4 for the selection of appropriate protocol.

Protocol A (96- and 384-well)

(Convert and detect protocol)

1. Reconstitute reagents and allow to equilibrate to room temperature.
2. Prepare a negative control well (final volume 40 µl).
3. Add 20 µl of PPiLight™ converting reagent to 40 µl of sample.
4. Incubate at room temperature for 30 min.
5. Add 20 µl of PPiLight™ detection reagent.
6. Incubate for 30 min.
7. Read luminescence (0.1 s integrated reading).

NOTE: Sample and reagent volumes can be adjusted by the user but should be kept in the same ratio.

Protocol B (96- and 384-well)

(Continuous kinetics protocol)

1. Reconstitute reagents and allow to equilibrate to room temperature.
2. Prepare a negative control well.
3. Add 20 µl of PPiLight™ converting and detection reagent mixture to each well.
4. Read kinetics (0.1 s integrated reading).

NOTE: sample and reagent volumes can be adjusted by the user but should be kept in the same ratio.

Interpretation of results

Due to the convenience of bioluminescent measurement the PPiLight™ assay offers an easy method for the detection of inorganic pyrophosphate. The bioluminescent signal produced by the luciferase is directly proportional to the amount of inorganic pyrophosphate present.

Main considerations /trouble shooting

Negative control

40 µl of reconstitution buffer (96-well plate) should be prepared as a negative assay control. The values generated by this sample should be subtracted from the assay results.

Integration time

A 0.1 s integration time is highly recommended due to the high light output of the assay. If adjustment is needed this should be kept in the range 0.1-1 second.

Interferences

The PPiLight™ assay has been designed to be resistant to DMSO up to a final concentration of 10% (v/v) and to many other interfering compounds. Intensely red colored compounds will quench light emission and this 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.

Plate recommendations

White walled plates suitable for luminescence need to be used. Black plates can be used if excess light is a problem.

High background levels

Reaction components should be free from ATP and pyrophosphate contamination. Use gloves (latex or equivalent) when handling the PPiLight™ assay kit.

References

Ghetta, A., Matus-Ortega, M., Garcia-Mena, J., Deho, G., Tortora, P., and Regonesi, M.E. (2004) Polynucleotide phosphorylase-based photometric assay for inorganic phosphate. Anal. Biochem **327(2)** 209-214.

Eriksson, J., Karomohamed, S. and Nyren, P. (2001) Method for real time detection of inorganic pyrophosphatase activity. Anal. Biochem. **293(1)** 67-70.

Ordering information

PPiLight™ Pyrophosphate Detection Kit

LT07-610	500 Test Kit

Related products

PDELight™ HTS cAMP phosphodiesterase kit

The PDELight™ assay kit has been designed for use in HTS to identify inhibitors of phosphodiesterase activity and IC_{50} determinations. It is a generic, homogeneous assay designed for use in 96, 384 or 1536 well microtitre plate formats. The assay is suitable for all cAMP dependent phosphodiesterases.

Ordering information

PDELight™ HTS cAMP phosphodiesterase kit

LT07-600	500 Test Kit

PKLight™ HTS protein kinase assay

The PKLight™ assay kit is a generic, homogeneous assay designed for the use in the HTS of kinase activity using 96, 384 or 1536 well microtiter plates. The kit contains all reagents necessary for the assay all that is required from the user is a kinase of interest, a suitable substrate, buffer and ATP. Extensive research has shown the assay suitable for serine / threonine, tyrosine and lipid kinases.

Ordering information

PKLight™ protein kinase assay kit

LT07-500	500 test kit
LT07-501	5000 test kit