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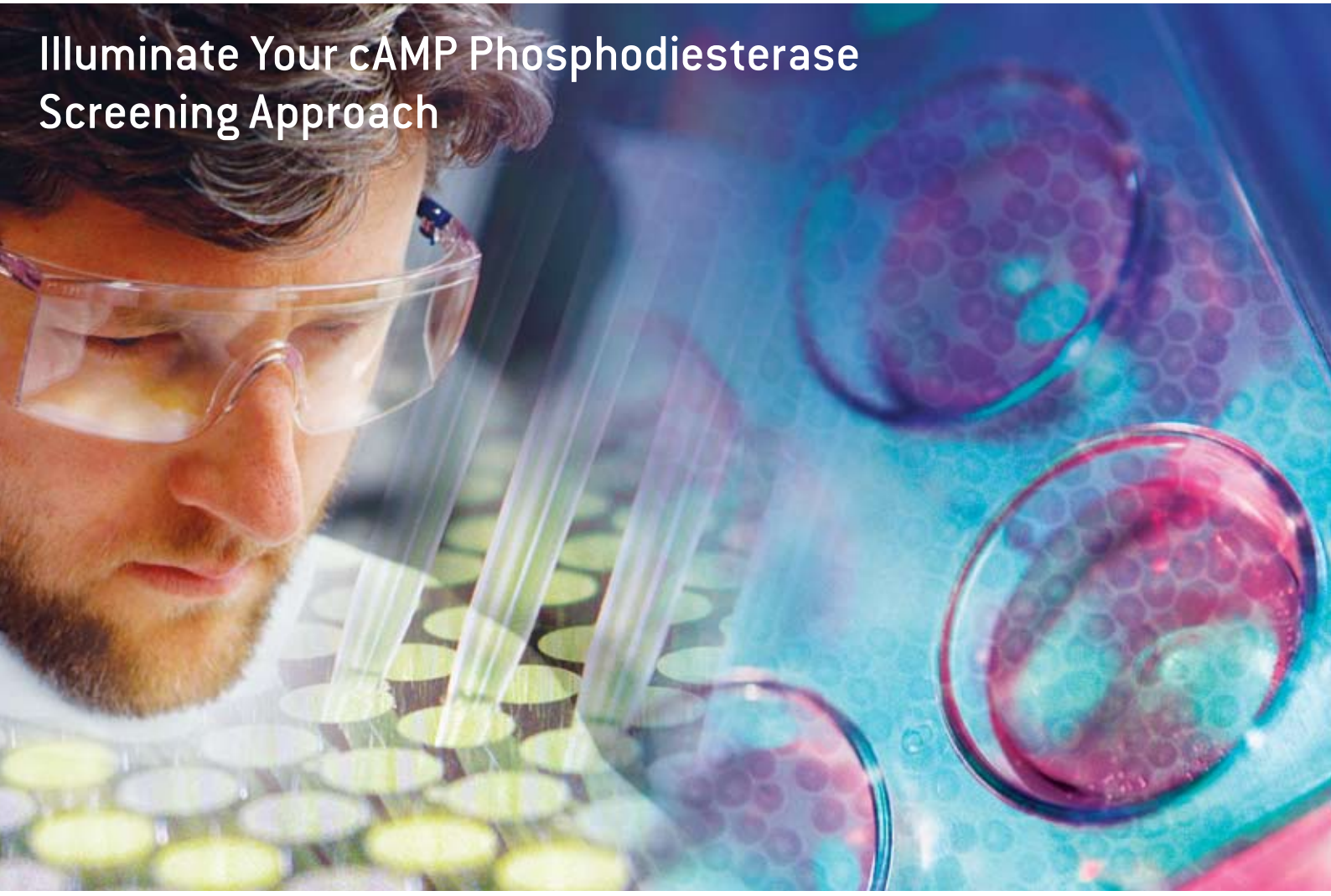
PDELight® HTS
cAMP Phosphodiesterase Assay Kit



PDELight® HTS cAMP Phosphodiesterase Assay Kit

The Power of Simplicity and Sensitivity

The PDELight® HTS cAMP Phosphodiesterase Assay Kit is a revolutionary new way to screen phosphodiesterases in high throughput applications. This assay is sensitive, robust and reproducible, and is a significant improvement over previous methods.

In the cell, phosphodiesterases function in conjunction with adenylate cyclase to regulate the amplitude of the ubiquitous second messenger signaling molecule, cyclic adenosine monophosphate (cAMP). Phosphodiesterases catalyze the hydrolysis of cAMP to adenosine monophosphate (AMP). There are at least 11 different families of phosphodiesterases, most of which contain more than one isozyme. Their substrate specificities, kinetics and tissue specific expression make phosphodiesterases druggable targets for a range of diseases.[1, 2]

A number of HTS assays are used to identify inhibitors of cAMP dependent phosphodiesterases. However, these assays suffer from a number of weaknesses, including the need for radioactivity, the use of beads, modified substrates, or antibodies, and are time consuming to perform.

The PDELight® Assay Kit is ideally suited for primary and secondary screening of cAMP dependent phosphodiesterases

- **Simple, Homogeneous Assay:** Add reagents directly to the completed PDE reaction and read
- **Generic Platform:** Use the same assay for all cAMP dependent phosphodiesterases; eliminate antibodies, radioactive beads, or modified substrates
- **Non-radioactive:** Non-hazardous reagents, no costly disposal costs
- **Rapid Assay:** Complete a 384-well plate in less than 15 minutes
- **Bioluminescent Sensitivity:** Use small amounts of enzyme in 96, 384, or 1536-well formats
- **Reproducible, Robust Assay:** Low false positive rates, typical Z´ values > 0.7, and few artifacts result in good clean hits

Focused Compound Library System

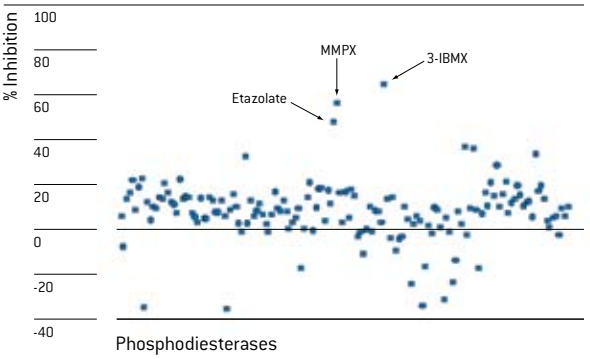


Figure 1. A focused library containing 150 pharmacologically active compounds (10 µM in 2.5% DMSO) was screened using the PDELight® Assay Kit protocol. The compounds, 3-IBMX, MMPX and Etazolate, inhibited 0.1 mU phosphodiesterase >50%.

IC_{50} Analysis

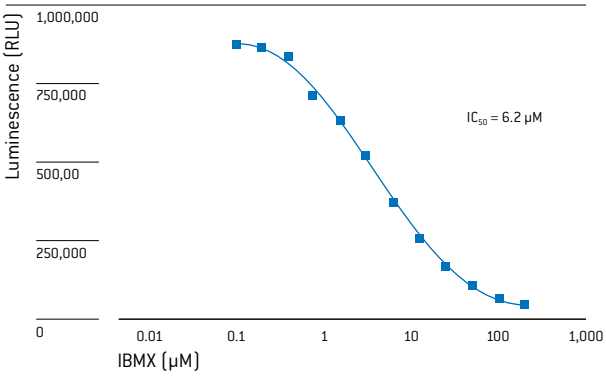


Figure 2. IC_{50} analysis of 3-IBMX = 6.2 µM.

Specifically Measure AMP with Exceptionally Loud Background

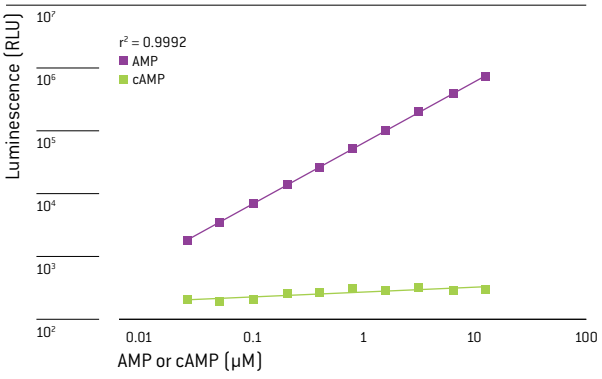


Figure 3. The PDELight® Assay Kit measures the AMP produced as a result of phosphodiesterase activity. The PDELight® Detection Reagent measures AMP up to 20 µM. The PDELight® Detection Reagent is specific for AMP only and not cAMP.

PDELight® HTS cAMP Phosphodiesterase Assay Kit

Direct Measurement of Reaction Product Yields Best Results

The PDELight® Assay Kit is a generic, homogeneous assay designed for use in high throughput screening of phosphodiesterase activity. It uses the power of bioluminescent detection to provide a simple alternative to existing phosphodiesterase assays with a generic endpoint determination for use with all cAMP dependent phosphodiesterases. The AMP produced from phosphodiesterase hydrolysis of cAMP is quantified using a robust and highly sensitive luciferase-based luminescent reagent. The AMP is directly converted to ATP and quantified as light. Nearly a photon of light is emitted for every molecule of ATP produced. The assay can easily be optimized for each phosphodiesterase to produce rapid, quality data suitable for IC_{50} determinations of screen compounds. The signal is glow luminescence with a half-life of greater than two hours, which is detected using a luminometer. The assay is extremely simple to use and can be run in several protocols to suit your needs.

The protocol (right) illustrates one of the procedures which can be used to measure *in vitro* phosphodiesterase activity or inhibition with the PDELight® Assay Kit.

The PDELight® Assay Kit is very effective in both primary and secondary screening approaches and can be employed to rapidly determine IC_{50} values in a library.

The PDELight® Assay Kit is very specific with exceptionally low backgrounds resulting in Z´ values greater than 0.7.

Unlike existing screening technologies such as IMAP® and Scintillation Proximity Assay (SPA™), the PDELight® Assay Kit does not require the use of expensive radioactive beads, radio-labeled cAMP or specially designed antibodies.

The PDELight® Assay Kit is supplied ready-to-use at a fraction of the cost of other assays.

Sample Protocol using the PDELight® Assay Kit

10 µl of Inhibitor (40 µM in 10% DMSO)
+
10 µl Phosphodiesterase
+
20 µl of cAMP Substrate (40 µM)
Incubate for 30 – 60 minutes at room temp
+
20 µl of PDELight® Detection Reagent
Incubate for 10 minutes at room temp
▼
Measure luminescence (0.1 – 1 second/well)

Table 1. Comparison of Phosphodiesterase Assays

	PDELight®	IMAP®	SPA™	HitHunter®
Radioactive	No	No	Yes	No
Requires beads	No	Yes	Yes	No
Substrate	cAMP	cAMP plus fluorescein	cAMP plus tritium	cAMP plus enzyme donor
Miniaturizable	96/384/1536	96/384/1536	96/384	96/384
Incubation Time	10 mins	1 hr	1 hr	1 hr

Ordering Information

Cat. No.	Description	Size
LT07-600	PDELight® HTS cAMP Phosphodiesterase Assay Kit	500 tests

For more information or for bulk reagent pricing, please contact your Lonza Sales Representative or HTS Specialist.

References

1. Conti M, Richter W, Mehats C, Livera G, Park JY, Jin C: Cyclic AMP-specific ODE4 phosphodiesterases as critical components of cyclic AMP signalling. *J. Biol. Chem.* (2003) **278**, 5493.
2. Conti M: Phosphodiesterases and cyclic nucleotide signalling in endocrine cells. *Mol. Endicrinol.* (2000) **14**, 1317.

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