AMP-Glo™ Assay

Sensitive Detection of AMP

Features

Well-suited for Low-activity Enzymes

Measure enzyme activity that more closely mimics physiological conditions.

Excellent Sensitivity

Assay is sensitive to low AMP concentrations, thus requires less enzyme than other assays, potentially lowering costs.

Works with Any AMP-Producing Enzyme

Enables screening of a wider range of targets.

Proven Luminescent Technology

Assay powered by Ultra-Glo™ Luciferase. No interference by fluorescent compounds.

Description

AMP-Glo™ Assay is a homogeneous assay that generates a luminescent signal from any biochemical reaction that produces AMP as a reaction product. This versatile system can measure the activity of a broad range of enzymes, such as cyclic AMP-specific phosphodiesterases, aminoacyl-tRNA synthetases, DNA ligases, ubiquitin ligases as well as enzymes modulated by AMP.

The AMP-Glo™ Assay is designed in a wide range of plate formats, including high-throughput formats. The stable luminescent signal of the assay allows batch-mode processing of multiple plates. The assay can be used to determine the AMP produced either in the presence or absence of ATP as a substrate.

Substrates for enzymes that do not use ATP.

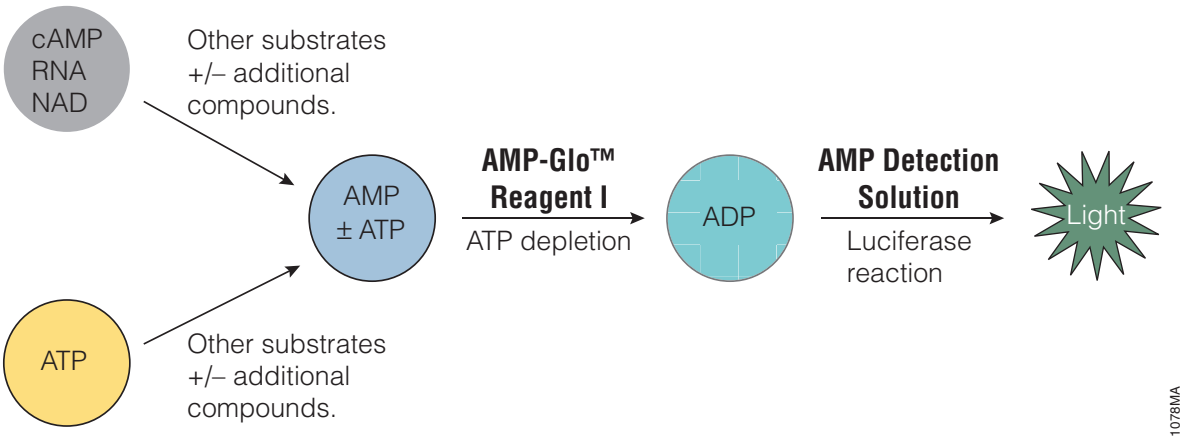


Figure 1. The AMP-Glo™ Assay can be used to detect activity of enzymes that catalyze any reaction that produces AMP as a reaction product, including enzymes that do not use ATP as a substrate (e.g., cAMP-specific PDE, polyA-deadenylases, ribonucleases, bacterial DNA ligase) as well as enzymes that use ATP as a substrate (e.g., ubiquitin ligase, aminoacyl tRNA synthetase, eukaryotic DNA ligase, succinyl CoA synthetase).

No Interference by ATP, cAMP

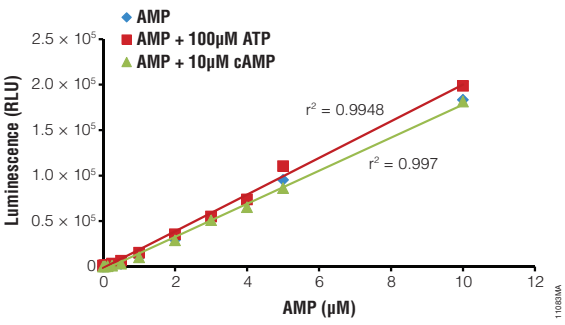
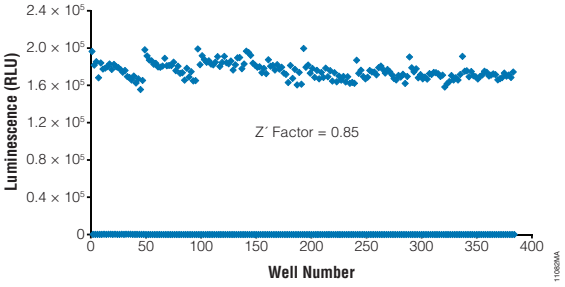


Figure 2. Titration of purified AMP. Reactions were assembled with the indicated concentrations of pure AMP in a low-volume, 384-well plate. AMP was titrated alone, with 100µM ATP or with 10µM cAMP. The AMP-Glo™ Assay was performed as described in Section 4.A of Technical Manual TM384. Data were collected using a plate-reading luminometer. Each point represents the average of four separate reactions, and error bars show standard deviation. Data analysis was performed using Excel®, Microsoft Office Software version 2007.

Excellent Z´ Values



IC_{50} Determinations using AMP-Glo™ Assay

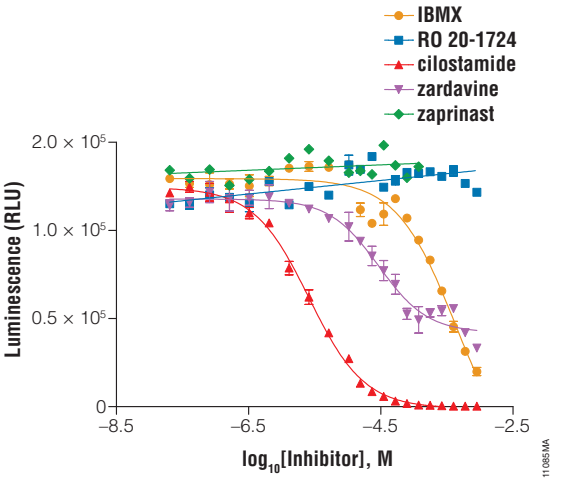


Figure 3. Determination of IC_{50} for various PDE inhibitors. The PDE reaction was performed with 20µM cAMP as a substrate in a solid white, low-volume 384-well plate. The PDE3B reaction was incubated 60 minutes at room temperature (23°C). The AMP-Glo™ Assay was performed as described in Section 4.C of Technical Manual TM384. Each point represents the average of three trials; the error bars show the standard deviation. Data analysis was performed with GraphPad Prism® software, version 4.02 for Windows

Figure 4. A scatter plot to determine the Z´ factor of the AMP-Glo™ Assay. PDE4B2 reaction at 0.7ng/reaction was run in the presence of 20µM cAMP for 60 minutes at room temperature in a low-volume 384-well plate. The AMP-Glo™ Assay was performed as described in Section 4.D of Technical Manual TM384 using the Deerac Fluidics® Equator™ HTS noncontact dispenser. Luminescence was measured using the PHERAstar high-end multiwell plate reader (BMG Labtech). Data analysis was performed using Sigma Plot/Sigma Stat, version 9.0 for Windows®.

For more information about AMP-Glo™ Assay, visit:
www.promega.com/DetectAMP

Ordering Information

Product	Size	Cat.#
AMP-Glo™ Assay	1,000 Assays	V5011
AMP-Glo™ Assay	10,000 Assays	V5012