Using luminescent ADP-Glo™ Max assay to detect activity modulators of transmembrane ATPases and ABC transporters

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1. Abstract

Transmembrane ATPases play an important role in either importing the many metabolites necessary for cell metabolism or exporting the toxins, waste, and drugs that can interfere with cellular processes. An important example of import proteins is the sodium-potassium pump (or Na+/K+ATPase), which maintains the ionic concentration balance that is required for cell viability. Due to its activity modulation by cardiac glycosides, Na+/K+ ATPase emerged as a drug target for heart-related conditions. Another example of transporters important in drug discovery is the family of ATP-binding cassette (ABC) transporters that are associated with multi-drug resistance (MDR). To ensure drug candidates will have good bioavailability and distribution and less toxicity, it is important to discern drug interaction with these transporters early in drug discovery. These drug efflux pumps are characterized by their ATPase activity that is both coupled to the transport activity and independent of substrate transport. ATPase activity of the ABC transporters is involved in two aspects: identifying how a drug affects it and identifying drug candidates that can interfere with ATPase activity of the transporter. ATPase activity or identifying how a drug affects it can lead to new therapeutic agents, or better understanding of the interaction, respectively. Because these transporters usually have a low ATP turnover rate and/or high ATP requirements, a sensitive assay is needed with high throughput for early drug screening efforts. The ADP-Glo™ Max luminescent ATPase detection assay provides a universal, homogeneous, and high-throughput method for measuring ATPase activity by quantifying the amount of ADP produced during the reaction. The assay is sensitive enough to detect low amounts of ADP in the presence of high amounts of ATP in a broad range of ATP concentrations (up to 5mM). This is of distinct advantage for drug transporters which typically have a high K_m for ATP. The luminescent signal generated is proportional to the ADP concentration produced and it correlates to the extent of ATPase activity, the decrease in the extent of ATPase activity, the decrease in the extent of ADP production and the inhibition of the transporters. ADP-Glo™ Max Assay can be used with virtually any ATPase and because of its sensitive-sensitivity, only a small amount of transporter membranes is required to achieve a high signal to background ratio. The ADP-Glo™ Max luminescent detection scheme also minimizes interference from reaction components that interfere with other detection schemes. This highly sensitive ready-to-use assay can easily replace the low throughput, significantly less sensitive calorimetric or fluorescent ATPase assays that are based on inorganic phosphate detection. A competition of the ADP-Glo™ Max assay to the luciferase assay showed that the luminescent assay has a lower level of detection allowing both a savings in enzyme usage and better assay performance to identify relevant chemical probes.

2. ADP-Glo™ is a positive detection Assay for product formation

○ Step 1: Deposition of unconsumed ATP after the ATPase reaction.
○ Step 2: ADP is converted into ATP that is detected via a luciferase/luciferin reaction.
○ Luminescent signal is proportional to ADP produced and the ATPase activity.

3. ADP-Glo™ Max can be used with a broad range of ATP concentrations

ADP conversion curves at different ADP/ATP concentrations

4. Comparison of detection limits of ADP and phosphate detection assays

Na+K+ ATPase activity detection using luminescent ATP assay compared to colormetric and Fluorescent Pi detection assays

ADP-Glo™ Max detects the activity of small amount of ATPase with high SB and high Signal to Noise

5. Screening inhibitors with ADP-Glo™ Max generates meaningful data

Screening the LOPAC library for Na+K+ ATPase inhibitors and compound interference in the ADP-Glo™ Max Assay

6. Stimulation of MDRs ATPase activity by reference compounds detected by ADP-Glo™ Max

ATPase assay using 5mM ATP and 10µg of membranes expressing different transporters

ADP-Glo™ Max detects high and low activity MDRs with low amount of membrane preparations (10µg)

7. Inhibition of basal and stimulated ATPase activity of MDRs detected by ADP-Glo™ Max

ADP-Glo™ Max assay is adequate for screening for MDRs inhibitors

8. General features of ADP-Glo™ Max assay

○ Universal: Any ATPase, e.g. Na+/K+ ATPase, ABC transporters (MDRs).
○ High dynamic range: High Signal to Background at low % ATP to ADP conversion allows use of lower amount of enzyme or membranes.
○ Broad range of ATP conc. (linear from µM to mM range) allows detection of activities requiring high Km for ATP like transporters.
○ Luminescent assay: Less Compound interference.
○ Homogeneous, non radioactive and Antibody Free
○ Robust Assay (Z’ higher than 0.7)
○ Convenient: For rapid high-throughput screening for Novel inhibitors of the Na+K+ pump and for both MDR transport substrates and inhibitors without the need for cell culture.

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