

Pgp-Glo™ Assay Systems

Features

- **Complete System:** The protocol and reagents of the Pgp-Glo™ Assay System have been tested for optimal performance. Systems available with and without p-glycoprotein.
- **Simplified Method:** The simple, homogeneous protocol makes the assay amenable to high-throughput screening in multiwell plates.
- **Low False-Positive Rate:** Use of a stabilized firefly luciferase (Ultra-Glo™ Luciferase) and a proprietary buffer formulation minimizes the incidence of false-positives due to inhibition of luciferase by analytes when screening for p-glycoprotein modulators.
- **Stable Activities:** Glow-type signal allows processing of multiple samples without concern for variability over time.
- **Mechanistic Assay:** The Pgp-Glo™ Assay reveals the capacity of a compound to stimulate or inhibit Pgp ATPase activity.

Description

The Pgp-Glo™ Assay Systems^(a,b) provide the necessary reagents for performing luminescent p-glycoprotein (Pgp) ATPase assays. Pgp, also known as MDR1 and ABCB1, is a 170kDa integral plasma membrane protein that functions as an ATP-dependent drug efflux pump and plays an important role in multi-drug resistance, drug absorption and drug-drug interactions. Compounds that interact with Pgp can be identified as stimulators or inhibitors of its ATPase activity. Compounds that are substrates for transport by Pgp typically stimulate its ATPase activity.

The Pgp-Glo™ Assay detects the effects of compounds on recombinant human Pgp in a cell membrane fraction. The assay relies on the ATP dependence of the light-generating reaction of firefly luciferase. ATP is first incubated with Pgp, then the Pgp ATPase reaction is stopped, and the remaining ATP is detected as a luciferase-generated luminescent signal (Figure 1). Pgp-dependent decreases in luminescence reflect ATP consumption by Pgp; thus the greater the decrease in signal the higher the Pgp activity. Accordingly, samples containing compounds that stimulate the Pgp ATPase will have significantly lower signals than untreated samples.

The luminescence detection portion of the Pgp-Glo System is catalyzed by a stabilized mutant of the firefly luciferase enzyme that provides stable, glow-type luminescent signals that allow a wide window of time for reading samples. After an initial 20-minute signal-stabilization period, readings can be taken immediately or after a delay (up to two hours) to give the same results in terms of calculated Pgp ATPase activity (Figure 3). In contrast, the colorimetric signals from molybdate-based ATPase assays are quickly saturated. Thus the luminescent approach allows users more time to perform reactions and simplifies automation of the assay.

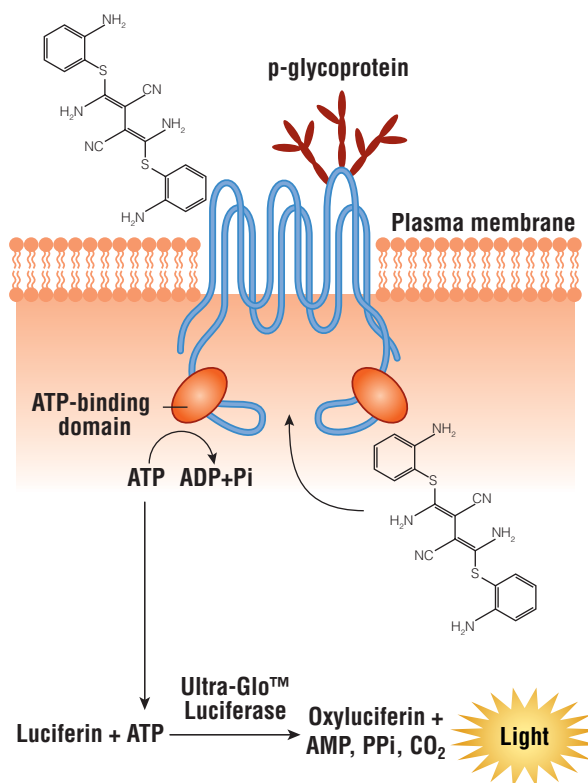


Figure 1. Pgp-Glo™ Assay System. Pgp is an ATP-dependent efflux pump for a wide range of drugs that plays an important role in multi-drug resistance, drug absorption and drug-drug interactions. Drugs that are transported by Pgp can be identified as stimulators of its ATPase activity. The Pgp-Glo™ Assay relies on the ATP-dependence of the light-generating reaction of firefly luciferase. After a pool of ATP is first exposed to the Pgp ATPase, ATP consumption is detected as a decrease in luminescence from a second reaction with a recombinant firefly luciferase (Ultra-Glo™ Luciferase).

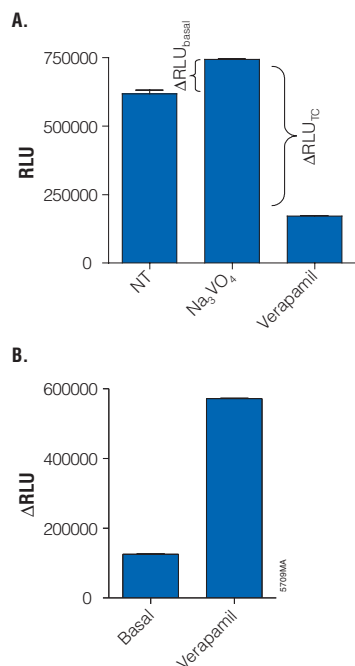


Figure 2. Stimulation of Pgp ATPase activity by Verapamil. Pgp reactions were performed plus and minus the Pgp inhibitor Na₃VO₄, without added drug (NT) or with 200mM Verapamil. The decrease in luminescence of NT samples compared to samples plus Na₃VO₄ (ΔRLU_{basal}) represents basal Pgp ATPase activity. The decrease in luminescence of Verapamil-treated samples (ΔRLU_{TC}) represents Verapamil-stimulated Pgp ATPase activity. ΔRLU_{basal} and ΔRLU_{TC} were replotted (Panel B) to illustrate the stimulation of Pgp ATPase activity by Verapamil.

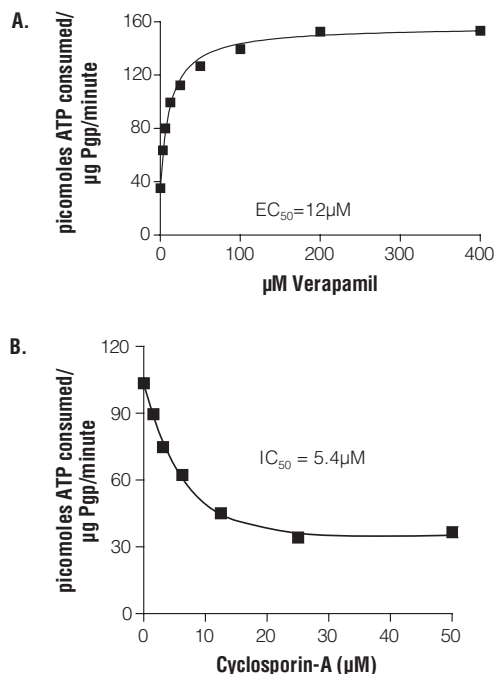


Figure 4. EC₅₀ and IC₅₀ measurements using the Pgp-Glo Assay. Panel A. Verapamil was tested at a range of concentrations for its capacity to stimulate Pgp ATPase activity. Panel B. IC₅₀ measurements where cyclosporin-A was tested at a range of concentrations for its capacity to inhibit 200μM Verapamil-stimulated Pgp ATPase activity.

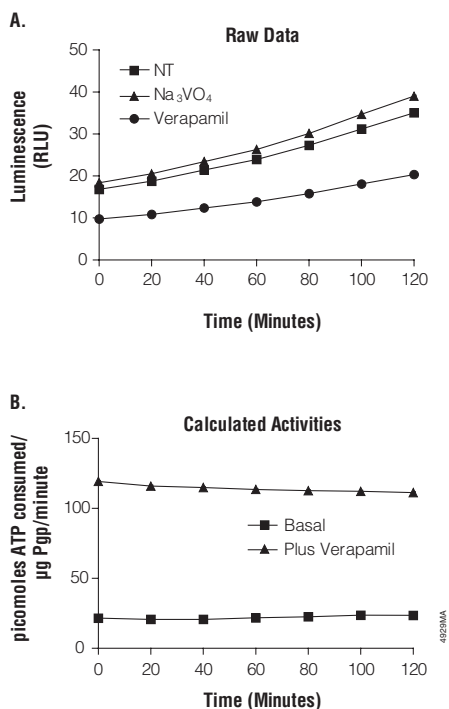


Figure 3. Stability of calculated activities. Untreated (NT), Verapamil-treated and Na₃VO₄-treated Pgp reactions were incubated for 40 minutes at 37°C in a 96-well plate. The reactions were stopped and luminescence initiated by addition of ATP Detection Reagent. Following a 20-minute room temperature signal-stabilization period (Time 0), luminescence was read. Luminescent readings were taken at 20-minute intervals for two hours. Raw data (Panel A) was converted to ATPase activities (Panel B) by comparing the luminescence of samples at each time point to luminescence of ATP standards at the corresponding time point.

Screening Pgp inhibition earlier in drug discovery

Studies to identify potential drug interactions and absorption profiles are a critical component of late-stage drug development. The availability of methods to predict properties of new chemical entities (NCEs) early in the discovery process enables the design of better medicines and reduces late-stage failures.

Benefits of testing the effects of compounds on Pgp activity early in the drug discovery process include: 1) The information can be used to alter the properties of NCEs so that compounds can be optimized and saved as potential therapeutic compounds; and 2) The assays can be used to screen and select NCEs with optimal activity profiles.

Ordering Information

Product	Size	Cat.#
Pgp-Glo™ Assay System	10ml	V3591
Pgp-Glo™ Assay System with P-glycoprotein	10ml	V3601

V3591: Contains sufficient reagents for 192 assays at 50μl per assay in 96-well plates.

V3601: Contains sufficient reagents for 96 assays at 50μl per assay in 96-well plates.

(a) The method of recombinant expression of *Coleoptera* luciferase is covered by U.S. Pat. Nos. 5,583,024, 5,674,713 and 5,700,673.

(b) Patent Pending.



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