The P450-Glo™ CYP2B6 Assay:
a rapid and selective assay for measuring CYP2B6 induction and inhibition

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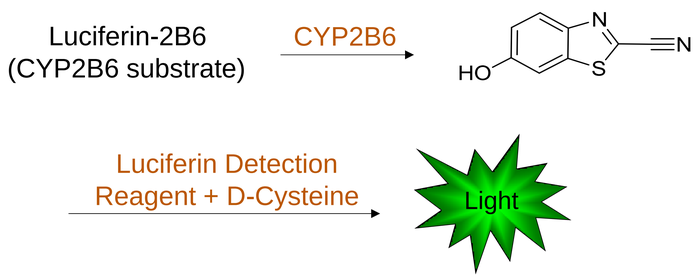
1. Introduction

We have developed a luminogenic CYP2B6 assay for biochemical CYP2B6 inhibition and for cell-based CYP2B6 induction studies. CYP2B6 is a cytochrome P450 expressed in human liver that metabolizes certain drugs and other xenobiotocs. CYP2B6 inhibitor and inducer drugs respectively slow or accelerate metabolism of co-administered CYP2B6 substrate drugs and can in this way contribute to adverse drug interactions. Our assay uses a probe substrate, 6-((4,4-dimethoxybutan-2-yl)oxy)benzo[d]thiazole-2-carbonitrile (Luciferin-2B6), which is selectively converted by CYP2B6 to a luciferin molecule that is detected as light output in a firefly luciferase reaction. The cell-based application of this assay can be easily combined with an ATP-based cell viability assay to derive CYP2B6 enzyme activity and cell viability measurements from the same well. The viability measurement reveals test article cytotoxicity and its impact on CYP2B6 activity. Here we present the CYP2B6 luminogenic assay characterization and demonstrate its utility for measuring time-dependent CYP2B6 inhibition, and for measuring CYP2B6 induction in cultured primary human hepatocytes with normalization to viable cell count.

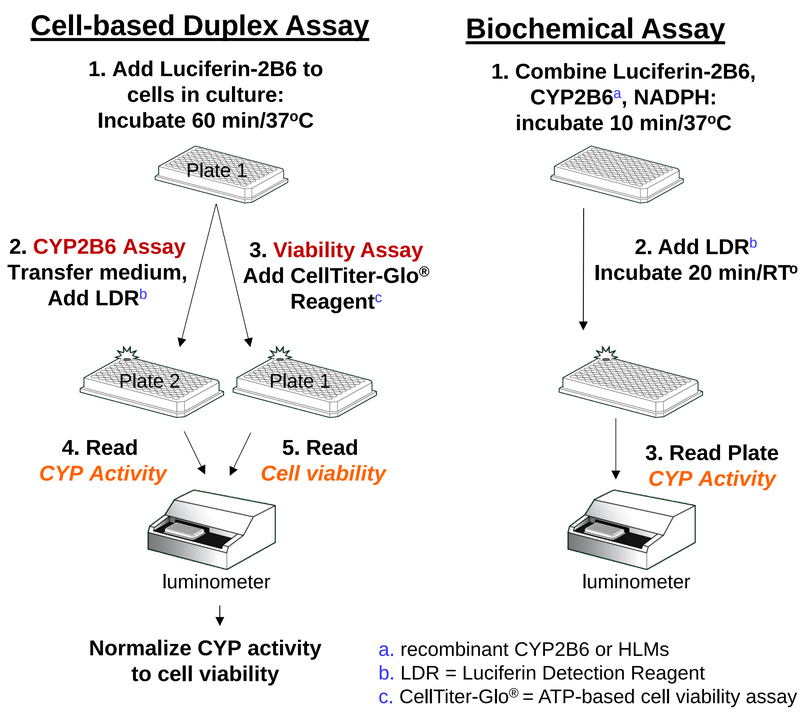
2. Bioluminescent CYP2B6 Assay Principle



CYP Activity Correlates with Light Intensity

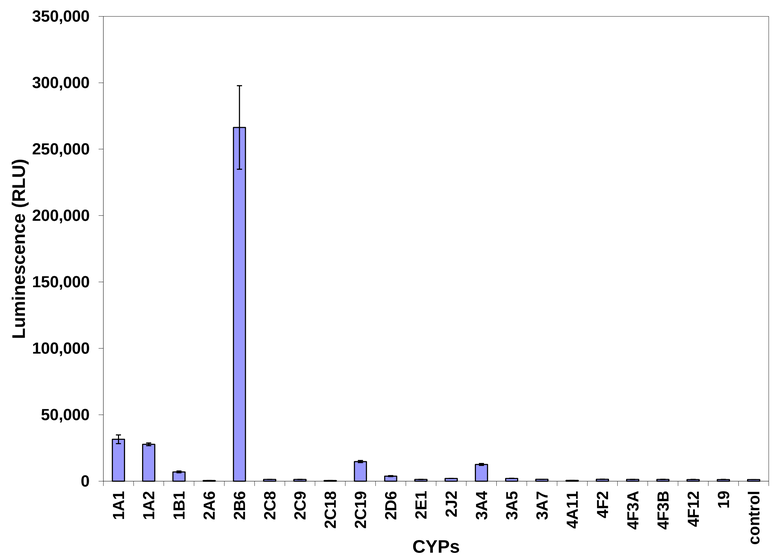
The assay uses the P450-Glo™ approach, which is based on the enzymatic release of a luciferin product from a non-reactive proluciferin compound. Light is produced as a result of the luciferase reaction in a Luciferin Detection Reagent. The amount of light correlates with the activity of the CYP enzyme in the first reaction.

3. Assay Protocols



a. recombinant CYP2B6 or HLMs
b. LDR = Luciferin Detection Reagent
c. CellTiter-Glo® = ATP-based cell viability assay

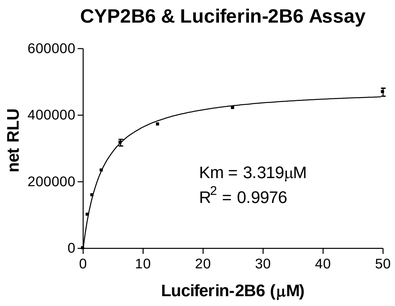
4. Luciferin-2B6 is selective for CYP2B6



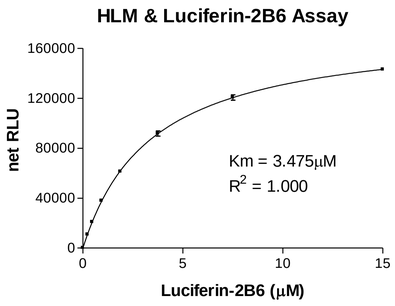
CYP Reactivity Profile of Luciferin-2B6

Equimolar amounts of recombinant human P450s were assayed for activity against Luciferin-2B6 using the biochemical P450-Glo™ method shown in panel 3 (mean ± SD, n=3).

5. Luciferin-2B6 Km with CYP2B6 and HLM



0.1pmol CYP2B6 per 50µL rxn was assayed for activity against Luciferin-2B6 by the biochemical P450-Glo™ method (panel 3) (mean ± SD, n=3).



1µg HLM per 50µL rxn was assayed for activity against Luciferin-2B6 using the biochemical P450-Glo™ method (panel 3) (mean ± SD, n=3).

6. CYP2B6 and HLM with Luciferin-2B6

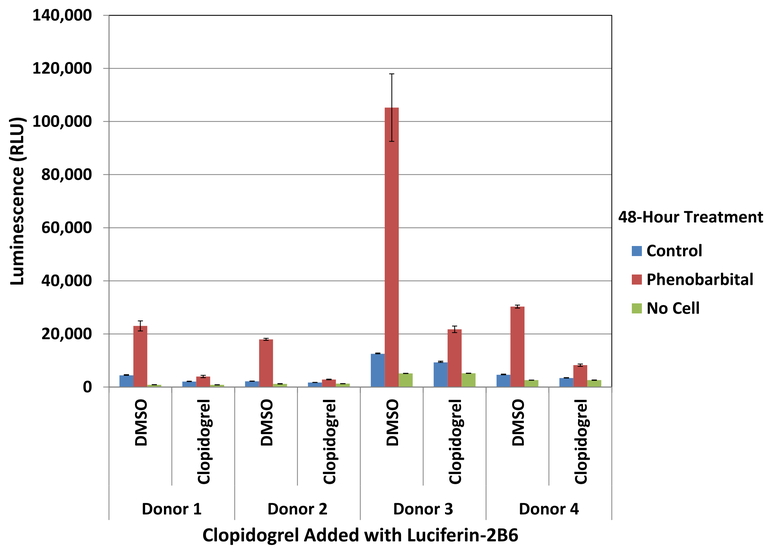
Time Dependence of CYP2B6-Selective Inhibitors

Inhibitor	HLM	HLM with preincubation	CYP2B6	CYP2B6 with preincubation
Clopidogrel	0.1675	0.06370	0.04038	0.02165
ThioTEPA	16.03	1.732	24.36	0.9725
Ticlopidine	0.2841	0.05673	0.1566	0.04383
Sertraline	12.67	0.6889	1.761	0.1481

IC_{50} values in µM.

0.1pmol CYP2B6 per 50µl / 1µg HLM per 50µl reactions were assayed for activity against Luciferin-2B6 and inhibitors, with and without 10-minute preincubation in the presence of NADPH using the P450-Glo™ method shown in the left lower.

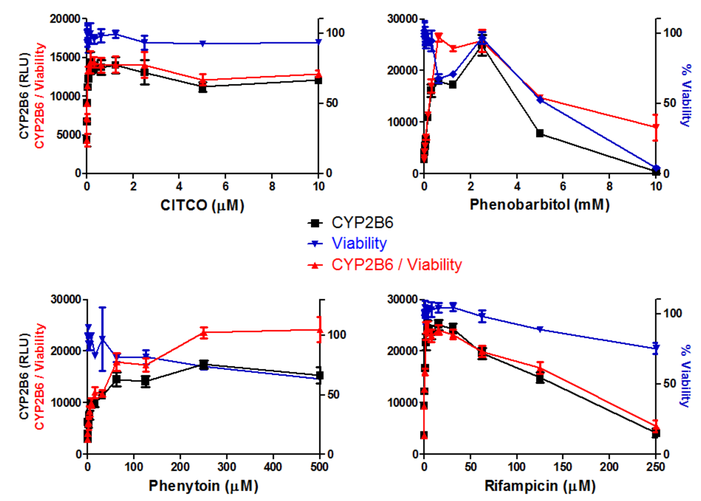
7. Human hepatocyte assay for CYP2B6 Induction with P450-Glo™



Human cryopreserved hepatocytes were treated with 1mM Phenobarbital for 48 hours before P450-Glo™ CYP2B6 assay as shown in the left lower (mean ± SD, n=3).

8. Duplex sssay of CYP2B6 cell viability

Human Cryopreserved Hepatocyte Assay with Luciferin-2B6 and Duplexed with CellTiter-Glo™



Human hepatocytes were treated with inducers for 48 hours before performing the cell-based duplex P450-Glo™ CYP2B6 and CellTiter-Glo® viability assay (panel 3) (mean ± SD, n=3).

9. Summary

The New P450-Glo™ CYP2B6 Induction/Inhibition Assay:

- Selective for CYP2B6.
- Enables a new P450-Glo™ CYP2B6 cell-based induction assay.
- One substrate for both cell-based and biochemical CYP2B6 applications.