Development of a Selective CYP2B6 and Cell Viability Duplex Assay

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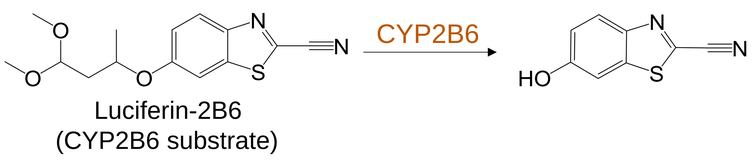
Abstract # 527

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 xenobiotics. 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.

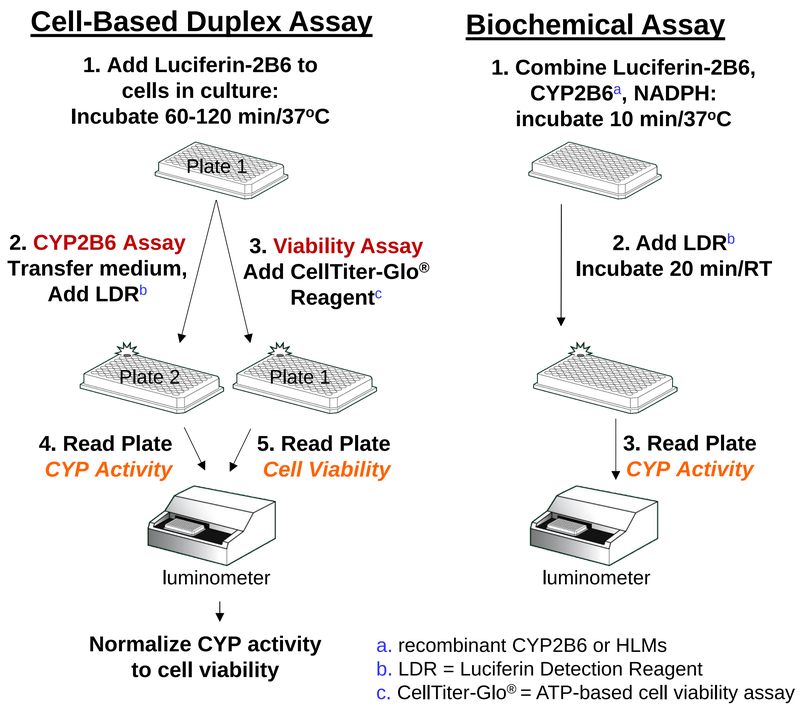
2. Bioluminescent CYP2B6 Assay Principle



CYP Activity Correlates with Light Intensity

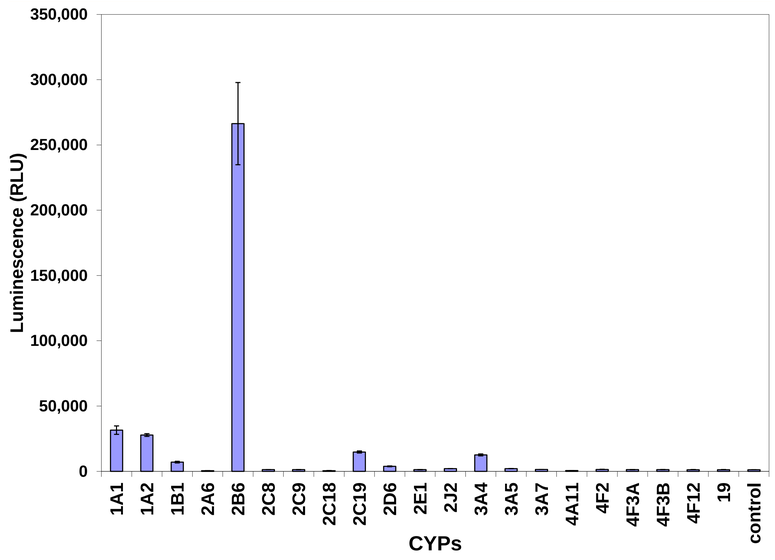
The assay uses the P450-Glo™ approach, which is based on the enzymatic release of a luciferin product from a proluciferin compound. Light is produced as a result of the luciferase reaction in a Luciferin Detection Reagent. The amount of light correlates with the activity of the CYP enzyme in the first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

3. Assay Protocols



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

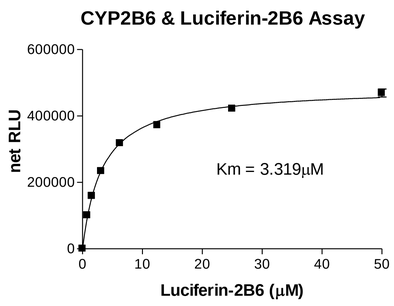
4. Luciferin-2B6 is Selective for CYP2B6



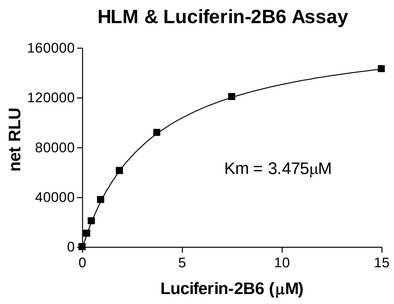
CYP Reactivity Profile of Luciferin-2B6

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

5. Luciferin-2B6 K_m with rCYP2B6 and HLM



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



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

6. CYP2B6 Inhibition Assays with Luciferin-2B6

Time Dependence of CYP2B6-Selective Inhibitors

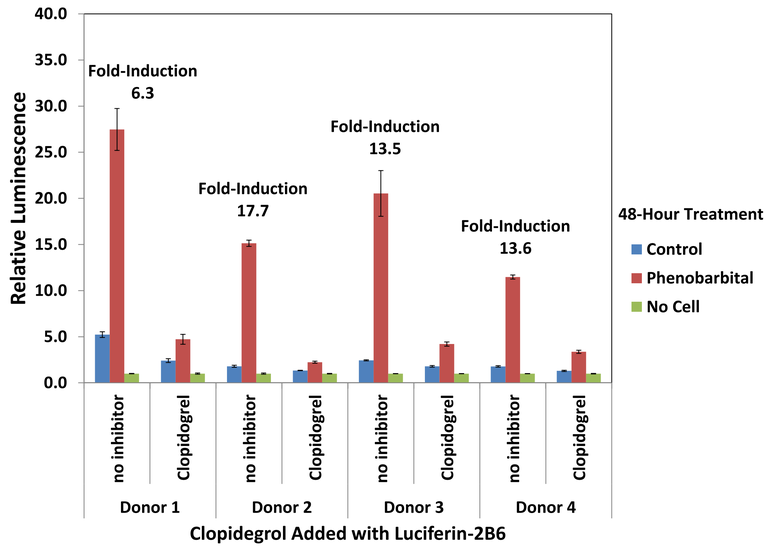
Inhibitor	HLM	HLM with preincubation	CYP2B6	CYP2B6 with preincubation
Clopidogrel	0.17 (0.046[1])	0.030	0.069	0.025
ThioTEPA	13.3 (1.75[2], 3.8[3], 8.3[1])	2.3	18.1	1.4
Ticlopidine	0.22 (0.13[3], 0.21[1], 0.32[2])	0.045	0.25	0.048
Sertraline	22.0 (3.2[4])	1.4	1.3	0.12

µM IC_{50} values are shown. Published values with conventional substrates are shown in parentheses.

Assays were configured at the apparent CYP2B6 K_m of Luciferin-2B6. 0.1pmol CYP2B6 or 1µg HLM per 50µl reactions were assayed for activity against Luciferin-2B6 and inhibitors, with and without 10-minute pre-incubation in the presence of NADPH using the biochemical P450-Glo™ method (see Panel 3).

[1]Walsky & Obach, DMD 35:2053-2059, 2007; [2]Turpeinen et al., DMD 32:626-631, 2004; [3]Obach et al., DMD 35:246-255, 2007; [4]Hesse et al., DMD 28:1176-1183, 2000.

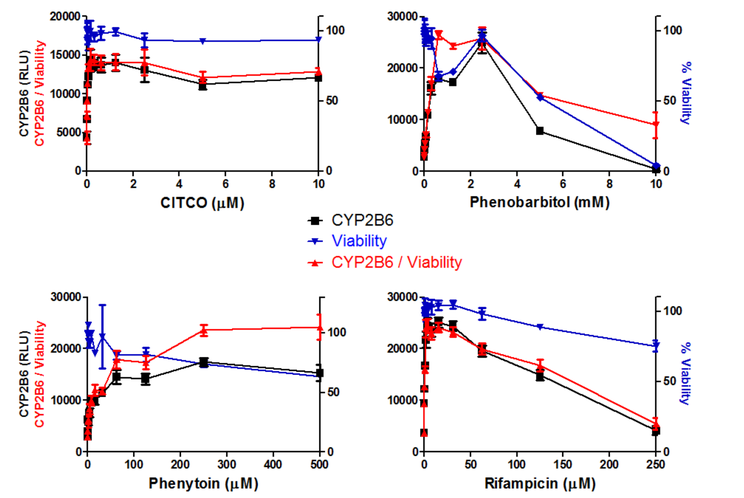
7. CYP2B6 Induction in Human Hepatocytes



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

8. Duplex Assay of CYP2B6 and Cell Viability

Human primary hepatocyte assays



Cryopreserved human hepatocytes were cultured and treated with inducers for 48 hours before performing the CYP2B6/CellTiter-Glo® duplex assay (see Panel 3) (mean ± SD, n=3).

9. Summary

Bioluminescent CYP2B6 Assay

- Selective for CYP2B6.
- Enables a new P450-Glo™ CYP2B6 cell-based induction assay.
- One CYP2B6 substrate enables both cell-based induction and biochemical inhibition applications.
- Compatible in duplex with CellTiter-Glo® cell viability assay.

March, 2014