P450-Glo™ Luminescent Cytochrome P450 Assays

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Abstract

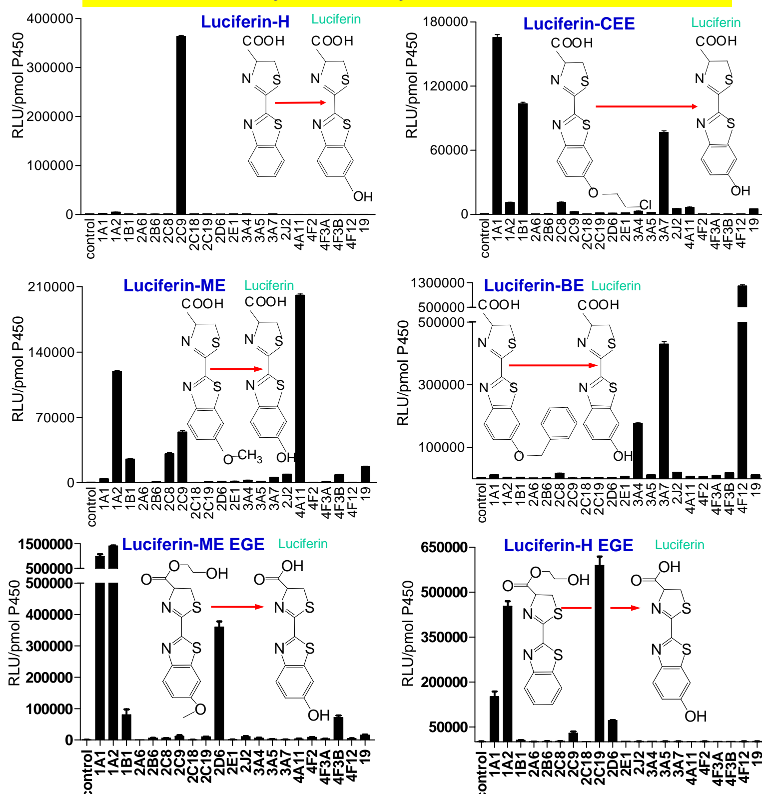
P450-Glo™ Luminescent P450 Assays utilize derivatives of beetle luciferin as luminogenic P450 enzyme substrates. Luciferin is a substrate for the light generating reaction of firefly luciferase. The derivatives are inactive with luciferase but are metabolized by P450s to luciferin, which in turn reacts with luciferase to make light. A homogenous luciferase mixture is added directly to P450 reactions that use luciferin derivatives as substrates. The amount of luciferin produced by a P450 is directly proportional to light output so light is used to measure P450 activity. Substrates are provided for CYP1A1, 1A2, 1B1, 2C8, 2C9, 2C19, 2D6, 2J2, 3A4, 3A7, 4A11, 4F3B, 4F12 and 19. P450-Glo Assays detect P450 activities in recombinant preparations, liver microsomes and cultured cells. P450-Glo™ Assays provide a simple, homogenous and robust luminescent assay format for rapid screening of multiple compounds against P450 enzyme activities.

P450-Glo™ Assay Scheme

General Assay Protocol

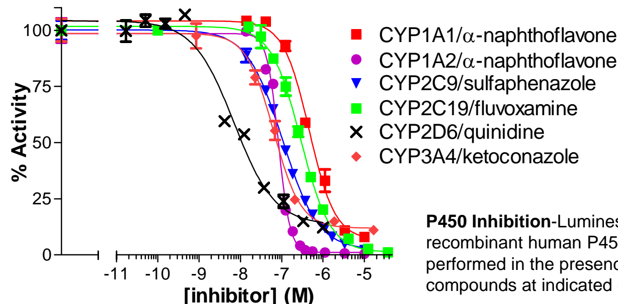
1. Prepare P450 reactions by combining a P450/P450 reductase sample, NADPH regenerating system and luciferin derivative in a multi-well plate format.
2. Incubate at room temperature or 37°C (e.g. 5-30 minutes).
3. Prepare luciferin detection reagent by combining the buffer and lyophilized luciferase/ATP cake provided with kits.
4. Terminate P450 reactions and start luminescent detection by adding one volume detection reagent to each P450 reaction well.
5. Read luminescence with a plate-reading luminometer.

P450 Enzyme Selectivity of Luciferin Derivatives



Recombinant human P450s were assayed for activity against Luciferin-H, Luciferin-ME, Luciferin-CEE, Luciferin-BE, Luciferin-ME EGE and Luciferin-H EGE using the P450-Glo™ method described above (mean±SD, n=3).

Using Luminescence To Measure CYP450 Inhibition



P450 Inhibition-Luminescent assays of recombinant human P450 enzymes were performed in the presence of inhibitory compounds at indicated concentrations.

IC_{50} Measurements From P450-Glo™ Assays With Recombinant Human P450s

CYP1A1/luciferin-CEE
Ketoconazole, 25.0
α-Naphthoflavone, 0.5
Phenacetin, 22.6
Quinidine, 3.2
Retinoic Acid, 18.0

CYP1A2/luciferin-ME
Azamulin, 31.4
Fluvoxamine, 0.12
Furafylline, 0.4
a-Naphthoflavone, 0.1
b-Naphthoflavone, 0.6

CYP2C8/luciiferin-ME
Ketoconazole, 82.6
Retinoic Acid, 2.0
Trimethoprim, 34.0

CYP2C9/luciferin-H
Azamulin, 26.4
Diclofenac, 2.4
Flurbiprofen, 65.5
Fluvoxamine, 9.8
Ibuprofen, 64.2
Naproxin, 161
Sulfaphenazole, 0.2
(S)-(-)-Warfarin, 4.6

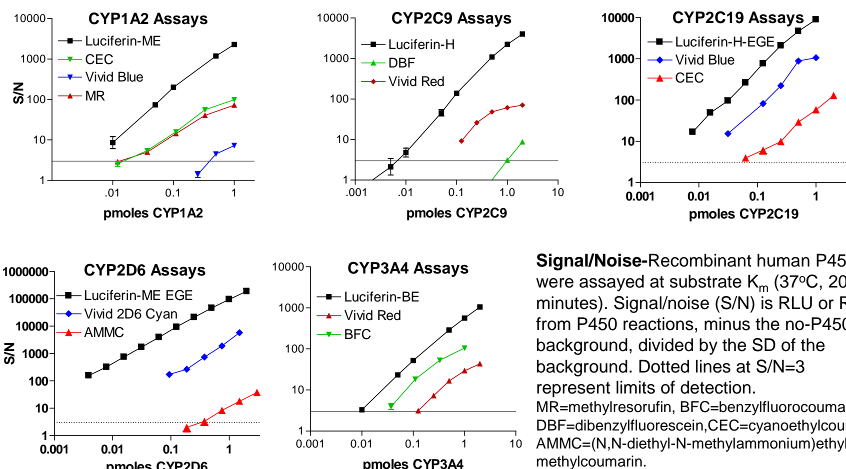
CYP2C19/lucif-H EGE
Azamulin, 26.8
Fluvoxamine, 0.3
Isoniazid, 76.2
Lansoprazole, 0.24
Mephenytoin, 68.2
Omeprazole, 2.4
Troglitazone, 4.2

CYP2D6/lucif-ME EGE
Azamulin, 39.2
Bufuralol, 22.8
Bupropion, 33.9
Clotrimazole, 22.6
Debrisoquine, 77.4
Dextromethorphan, 6.8
Fluoxetine, 6.0
Fluvoxamine, 3.4
Haloperidol, 1.2
Nicardipine, 8.0
Pindolol, 76.0
Quinidine, 0.01
Quinine, 11.0
Terfenidine, 3.6
Verapamil, 67.0

CYP3A4/luciferin-BE
Azamulin, 0.1
Clotrimazole, 0.006
Disopyramide, 29.0
Diltiazem, 0.5
Erythromycin, 1.2
Fluvoxamine, 15.2
Haloperidol, +
Ketoconazole, 0.1
Midazolam, 17.4
a-Naphthoflavone, +
Nifedipine, +
Omeprazole, 61
Testosterone, +
Troleandomycin, 0.2
Verapamil, 0.4

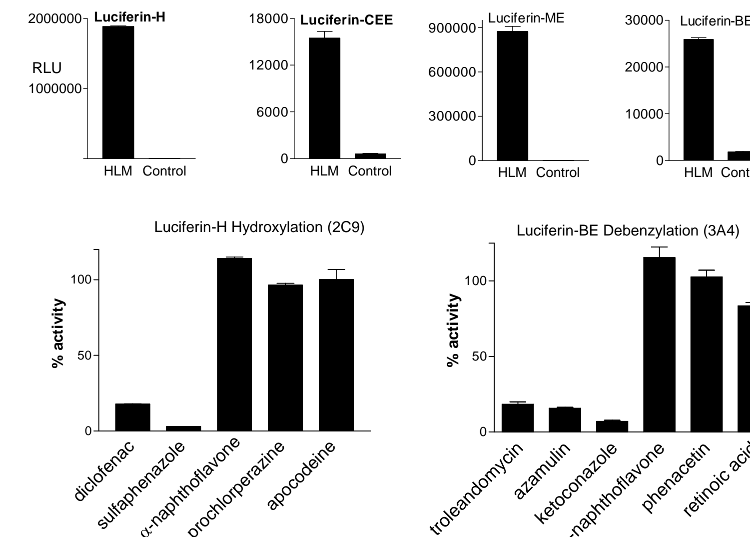
The number to the right of each compound is the IC_{50} (μM).
"+" indicates positive cooperativity (stimulation).

Assay Sensitivity: Luminescent > Fluorescent



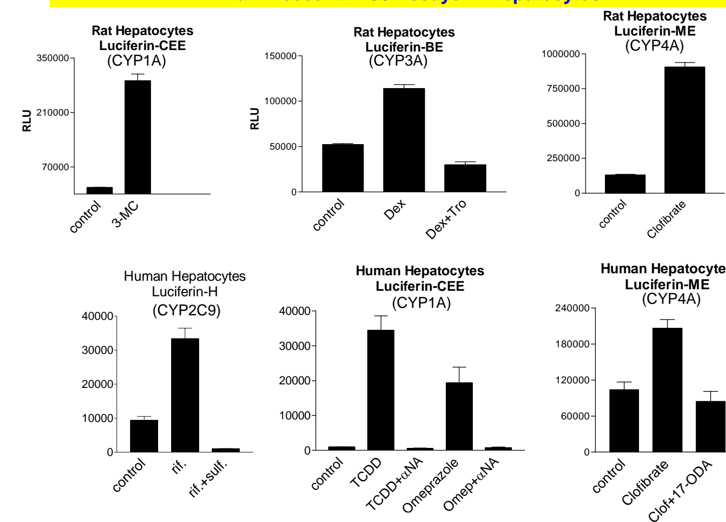
Signal/Noise-Recombinant human P450s were assayed at substrate K_m (37°C, 20-30 minutes). Signal/noise (S/N) is RLU or RFU from P450 reactions, minus the no-P450 background, divided by the SD of the background. Dotted lines at S/N=3 represent limits of detection.
MR=methylresorufin, BFC=benzylfluorocoumarin, DBF=dibenzylfluorescein,CEC=cyanoethylcoumari, AMMC=(N,N-diethyl-N-methylammonium)ethyl-m-methylcoumarin.

Luminescent P450 Assays in Human Liver Microsomes



P450-Glo™ reactions were performed with 20μg human liver microsomes (30 minutes at 37°C). Controls are insect cell membranes devoid of P450 activity. Activity was detected with each of four P450-Glo™ substrates (upper panels). The inhibition of luciferin-H hydroxylation by the CYP2C9-selective inhibitors diclofenac and sulfaphenazole indicates that CYP2C9 is the main luciferin-H hydroxylase and the inhibition of luciferin-BE debenzylation by the CYP3A4-selective inhibitors troleandomycin, azamulin and ketoconazole indicates that CYP3A4 is the main luciferin-BE debenzylase in liver microsomes (lower panels).

Luminescent P450 Assays In Hepatocytes



Rat or human hepatocytes were cultured as adherent monolayers and treated for 48 hours with inducers (3-methylcholanthrene (3-MC), dexamethasone (Dex), rifampicin (Rif), clofibrate, TCDD, omeprazole). Luciferin-CEE, luciferin-ME, luciferin-H or luciferin-BE were then added to cultures for 4 hours. Inhibitors, sulfaphenazole (sulf), α-naphthoflavone (αNA), 17-octadecynoic acid (17-ODA)) were coincubated with substrates. A sample of culture medium was then combined with an equal volume of luciferin detection reagent and luminescence was read on a luminometer.

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