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Protocol

P450-Glo™ Assay Technical Bulletin #TB325
(www.promega.com/tbs/tb325/tb325.html)

P450-Glo™ Screening SystemsTechnical Bulletin #TB325
(www.promega.com/tbs/tb325/tb325.html)

Ordering Information

Product	Size	Cat.#
P450-Glo™ CYP3A4 Assay with Luciferin-IPA	10 ml	V9001
	50ml	V9002
P450-Glo™ CYP3A4 Screening System with Luciferin-IPA		V9920

[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. A license (from Promega for research reagent products and from The Regents of the University of California for all other fields) is needed for any commercial sale of nucleic acid contained within or derived from this product.

More Cytochrome P450 Substrates!

SIX BIOLUMINESCENT SUBSTRATES FOR P450 ENZYME ASSAYS

A series of articles in *eNotes* describe six new substrates available from Promega for bioluminescent CYP assays.

Luminogenic CYP assays use prosubstrates for the light-generating reaction of firefly luciferase. CYPs convert the prosubstrates to an active luciferin, which produces light in a second reaction with luciferase. The amount of light generated is proportional to the amount of luciferin produced by the CYP and, therefore, to CYP enzyme activity.

LUCIFERIN-4A

CYP4A enzymes are cytochromes P450 that catalyze the ω-hydroxylation of fatty acids and the formation of arachidonic acid metabolites. Genes encoding CYP4A enzymes are induced by agonists of the peroxisome proliferator-activated receptor alpha (PPARα) nuclear receptor. CYP4A assays, including human CYP4A11 and rat CYP4A1, CYP4A2 and CYP4A3, commonly require chromatographic steps or cell lysate preparations that limit ease-of-use and throughput.

The luciferin derivative, 2-(6-methoxyquinolin-2-yl)-4,5-dihydrothiazole-4-carboxylic acid, referred to as Luciferin-4A, is selectively converted to quinolylluciferin, an active alternative to native beetle luciferin, by the human CYP4A11 enzyme. The eNotes article demonstrates use of the substrate in a luminogenic CYP4A11 biochemical assay and also in a cell-based assay that measures CYP4A basal and induced activity in intact rat hepatocytes.

See more:
www.promega.com/enotes/applications/ap0092.htm

LUCIFERIN-4F2/3

The cytochromes P450 CYP4F2 and CYP4F3B catalyze ω-hydroxylation of fatty acids and arachidonic acid and metabolize certain drugs. Enzyme assays for CYP4F2 and CYP4F3B typically include a chromatographic separation step that limits ease-of-use and throughput.

The luciferin derivative, 2-(6-(4-(methylthio)benzyloxy) benzo[d]thiazol-2-yl)-4,5-dihydrothiazole-4-carboxylic acid, referred to as Luciferin-4F2/3, is shown to have a high degree of selectivity for CYP4F3B and CYP4F2.

See more:
www.promega.com/enotes/applications/ap0093.htm

LUCIFERIN-4F12

CYP4F12 is a cytochrome P450 enzyme that catalyzes the oxidation of leukotrienes, arachidonic acid and certain drugs. CYP4F12 is expressed predominantly in the liver with lower levels in the kidney, colon, small intestine and heart. Enzyme assays for CYP4F12 typically include a chromatographic separation step (e.g., radiometric, HPLC or LC/MS-based assays), limiting the ease-of-use and throughput.

The luciferin derivative, 2-(6-(4-chlorobenzyloxy) benzo[d]thiazol-2-yl)-4,5-dihydrothiazole-4-carboxylic acid, referred to as Luciferin-4F12, is selectively converted to luciferin by the human CYP4F12 enzyme. The eNotes article demonstrates use of this luciferase prosubstrate in a luminogenic CYP4F12 biochemical assay to detect CYP4F12 activity and CYP4F12 inhibitors

See more:
www.promega.com/enotes/applications/ap0094.htm

LUCIFERIN-3A7

CYP3A enzymes are cytochromes P450 that oxidize many endogenous compounds, drugs and other xenobiotics. CYP3A7 is the predominant CYP3A enzyme in fetal and neonatal liver. CYP3A7 expression declines after birth in parallel with a dramatic increase in CYP3A4 and CYP3A5, which become the most abundant CYPs in adult liver and intestine. CYP3A7 assay chemistries are frequently cross-reactive with CYP3A4 and CYP3A5 and commonly require chromatographic steps that limit the assays in terms of ease-of-use and throughput.

The bis-luciferin, 2,2'-(6,6'-(1,3-phenylenebis (methylene))bis(oxy)bis(benzo[d]thiazole-6,2-diyl))bis(4,5-dihydrothiazole-4-carboxylic acid), referred to as Luciferin-3A7, is selectively converted to luciferin by the human CYP3A7 enzyme with little or no detectable activity with CYP3A4 or CYP3A5. The eNotes article demonstrates use of this luciferase prosubstrate in a luminogenic CYP3A7 biochemical assay to detect CYP3A7 activity and CYP3A7 inhibitors.

See more:
www.promega.com/enotes/applications/ap0095.htm

LUCIFERIN-2J2/4F12 (ESTER)

CYP2J2 is a cytochrome P450 enzyme (CYP) that catalyzes the formation of arachidonic acid metabolites that in turn regulate blood pressure, cell proliferation and inflammation. CYP2J2 is expressed most prominently in the human heart, but it also contributes to the intestinal metabolism of certain drugs. Enzyme assays for CYP2J2 typically include a chromatographic separation step that limits ease-of-use and throughput.

The luciferin derivative, 2-hydroxyethyl 2-(6-(3,3-dimethoxypropoxy)benzo[d]thiazol-2-yl)-4,5-dihydrothiazole-4-carboxylate, referred to as Luciferin-2J2/4F12, is converted to a luciferin ester, most prominently by CYP2J2 and to a lesser extent by CYP4F12, CYP1A1, CYP1B1 and CYP3A4. The Luciferin-2J2/4F12 substrate is selective for human CYP2J2, CYP4F12, CYP3A4, CYP1A1 and CYP1B1, and has a strong preference for CYP2J2 under conditions that favor activity of this enzyme.The eNotes article demonstrates use of this substrate in luminogenic CYP2J2 enzyme assays for detecting CYP2J2 activity and inhibitors .

See more:
www.promega.com/enotes/applications/ap0096.htm

PROMISCUOUS CYP SUBSTRATE: LUCIFERIN-MULTICYP (ESTER)

The cytochromes P450 (CYPs) are a large family of enzymes that catalyze the oxidation of numerous hydrophobic chemicals, including endogenous compounds, therapeutic drugs and environmental toxins. Known CYP substrates are used as probes for determining how a compound might interact with CYPs as substrate or regulator. A highly selective probe substrate is desirable when targeting a single CYP in a mix of other potentially cross-reacting enzymes. In contrast, a promiscuous probe substrate is useful for applications such as characterizing multiple CYP enzymes in isolation, measuring net CYP activity in a mixture, or for mutagenesis studies that can benefit from a substrate with loose active-site-binding constraints.

The promiscuous CYP substrate, methyl 2-(6-methoxybenzo[d]thiazol-2-yl)-4,5-dihydrothiazole-4-carboxylate, referred to as Luciferin-MultiCYP, reacts with at least 21 CYP enzymes.

See more:
www.promega.com/enotes/applications/ap0097.htm