CREATIVE BIOMART®
Assay Kit

Cytochrome P450 2C19 Fluorometric Activity Assay Kit

Product Information

Cat.No.

Kit-2124

Product Overview

CYP2C19 Activity Assay Kit enables rapid measurement of native or recombinant CYP2C19 activity in biological samples such as liver microsomes. The assay utilizes a non-fluorescent CYP2C19 substrate that is converted into a highly fluorescent metabolite detected in the visible range (Ex/Em = 406/468 nm), ensuring a high signal-to-background ratio with little interference by autofluorescence. A highly-selective CYP2C19 inhibitor is provided for determination of CYP2C19 activity in heterogeneous biological samples, where other CYP isozymes may contribute to substrate metabolism. The inhibitor displays greater than 100-fold selectivity for CYP2C19 over other CYPs, ensuring targeted inhibition. CYP2C19 specific activity is calculated by running parallel reactions in the presence and absence of the selective inhibitor and subtracting any residual activity detected with the inhibitor present. The kit contains a complete set of reagents sufficient for performing 50 sets of paired reactions (in the presence and absence of inhibitor).

Size

100 assays

Description

Cytochrome P450 2C19 (CYP2C19, EC 1.14.14.1) is a member of the cytochrome P450 monooxidase (CYP) family of microsomal xenobiotic metabolism enzymes. CYPs are membrane-bound hemeproteins responsible for Phase I biotransformation reactions, in which lipophilic drugs and other xenobiotic compounds are converted to more hydrophilic products to facilitate excretion from the body. CYP2C19 is primarily expressed in liver and intestinal tissue and catalyzes oxidation of neutral or weakly basic lipophilic molecules with 2- 3 hydrogen bond donor/acceptor sites. Isoforms of the CYP2C subfamily are responsible for metabolism of nearly 20% of all small molecule drugs commonly used by humans. Polymorphisms in the human CYP2C19 gene have been implicated in clinical drug/drug interactions involving widely-prescribed drugs, including proton pump inhibitors, antiplatelet agents and anticonvulsants. In addition, for drugs whose pharmacological activity

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requires metabolism from a pro-drug form, CYP2C19 inhibition or allelic deficiency can lead to decreased drug efficacy.

Applications

Rapid assessment of native/recombinant CYP2C19 activity in lysates or microsomal fractions prepared from tissues, cells.Screening of drugs and novel ligands for interaction with native/recombinant CYP2C19.

Target Species

Eukaryotes

Storage

Store kit at -20°C and protect from light. Briefly centrifuge all small vials prior to opening. Allow the CYP2C19 Assay Buffer to warm to room temperature prior to use. Read entire protocol before performing the assay procedure.3-CHC Standard: Reconstitute in 110 µl of DMSO and vortex until fully dissolved to yield a 5 mM stock solution. The 3-CHC stock solution should be stored at -20°C and is stable for at least 3 freeze/thaw cycles.CYP2C19 Inhibitor ((+)-N-3-benzylnirvanol): Reconstitute in 55 µl of acetonitrile and vortex until fully dissolved to yield a 6 mM stock solution. The stock solution is stable for 2 months at -20°C. To obtain a 150 µM working solution of (+)-N-3-benzylnirvanol (5X final concentration), add 25 µl of the 6 mM stock solution to 975 µl of CYP2C19 Assay Buffer. The 150 µM working solution should be stored at -20°C and used within one month (stable for at least 3 freeze/thaw cycles).NADPH Generating System (100X): Reconstitute with 220 µl CYP2C19 Assay Buffer, aliquot and store at -20°C. Avoid repeated freeze/thaw cycles and keep on ice while in use.β-NADP+ Stock (100X): Dissolve in 110 µl CYP2C19 Assay Buffer and vortex thoroughly to yield a 10 mM solution of NADP+ (100X stock). Store at -20°C, stable for at least 3 freeze/thaw cycles.CYP2C19 Substrate: Reconstitute with 110 µl anhydrous reagent-grade acetonitrile and vortex until fully dissolved to obtain a 5 mM stock solution. Store at -20°C. Allow the vial to warm to room temperature before opening and promptly retighten cap after use to avoid absorption of airborne moisture.Recombinant Human CYP2C19: Do not reconstitute until ready to use. Reconstitute with 230 µl CYP2C19 Assay Buffer and add 20 µl of NADPH Generating System (100X). Mix thoroughly to ensure a homogenous solution (the solution will have a slightly opaque, milky appearance), aliquot and store at -80°C. Avoid repeated freeze/thaw cycles and use aliquots

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within one month (the Recombinant Human CYP2C19 will lose approximately 10% activity per week when stored at -80°C). Thaw aliquots rapidly at 37°C and place on ice until use (thawed aliquots should be used within 4 hours).

Kit Components

CYP2C19 Assay Buffer: 100 ml3-CHC Standard: 1 viaCYP2C19 Inhibitor ((+)-N-3-benzylnirvanol): 1 viaNADPH Generating System (100X): 1 viaβ-NADP+ Stock (100X): 1 viaCYP2C19 Substrate: 1 viaRecombinant Human CYP2C19: 1 via

Detection method Fluorescence (Ex/Em 406/468 nm)

Compatible Sample Types

• Human liver microsomes and liver S9 fractions• Lysates of tissues and cultured cells, primary hepatocytes• Heterologously expressed recombinant CYP2C19 preparations

Features & Benefits

• Simple, convenient, highly sensitive • Fluorescent assay enables easy determination of CYP2C19 activity in a variety of biological samples • The substrate shows low background and a high signal-to-noise ratio • Kit includes CYP2C19 inhibitor (+)-N-3-benzylnirvanol and a stable, recombinant human CYP2C19 co-expressed with NADPH Reductase as a positive control