CREATIVE BIOMART®
Assay Kit

Cytochrome P450 2C9 Activity Fluorometric Assay Kit

Product Information

Cat.No. Kit-2116

Product Overview

CYP2C9 Activity Assay Kit enables rapid measurement of native or recombinant CYP2C9 activity in biological samples such as liver microsomes. The assay utilizes a non-fluorescent CYP2C9 substrate that is converted into a highly fluorescent metabolite detected in the visible range (Ex/Em = 415/502 nm), ensuring a high signal-to-background ratio with little interference by autofluorescence. A highly selective irreversible CYP2C9 inhibitor is provided for determination of CYP2C9 activity in heterogeneous biological samples, where other CYP isozymes may contribute to substrate metabolism. The inhibitor displays greater than 100-fold selectivity for CYP2C9 over other CYPs, ensuring targeted inhibition. CYP2C9 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 2C9 (CYP2C9, EC 1.14.14.1) is a member of the cytochrome P450 monooxidase (CYP) family of microsomal xenobiotic metabolism enzymes. CYP2C9 is primarily expressed in the liver and catalyzes oxidation of small, weakly acidic or hydrophobic molecules containing an aromatic moiety and at least one hydrogen bond donor. Isoforms of the CYP2C subfamily are responsible for metabolism of nearly 20% of all small molecule drugs commonly used by humans. The human CYP2C9 gene is highly polymorphic and CYP2C9 single nucleotide polymorphisms (SNPs) have been implicated in clinical drug/drug interactions involving widely-prescribed drugs with narrow therapeutic indices such as warfarin, phenytoin and indomethacin. In addition, for drugs whose pharmacological activity requires metabolism from a pro-drug form (such as the antihypertensive drug losartan), CYP2C9 inhibition or allelic deficiency can lead to decreased drug efficacy.

Tel: 1-631-559-9269 1-516-512-3133
Email:info@creative-biomart.org
Fax:1-631-938-8127
45-1 Ramsey Road, Shirley, NY 11967, USA

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Applications

Rapid assessment of native/recombinant CYP2C9 activity in lysates or microsomal fractions prepared from tissues, cells.Screening of drugs and novel ligands for interaction with native/recombinant CYP2C9.

Storage

Store kit at -20°C and protect from light. Briefly centrifuge all small vials prior to opening. Allow the CYP2C9 Assay Buffer to warm to room temperature prior to use. Read entire protocol before performing the assay procedure.7-HFC Standard: Reconstitute in 110 µl of DMSO and vortex until fully dissolved to yield a 5 mM stock solution. Store at -20°C, stable for at least 3 freeze/thaw cycles.CYP2C9 Inhibitor (Tienilic Acid): Reconstitute in 55 µl of acetonitrile and vortex until fully dissolved to yield a 20 mM stock solution. To obtain a 300 µM working solution of tienilic acid (5X final concentration), add 15 µl of the 20 mM stock solution to 985 µl of CYP2C9 Assay Buffer. The 300 µM working solution should be stored at -20°C and is stable for 3 freeze/thaw cycles. The stock solution is stable for 2 months at -20°C.NADPH Generating System (100X): Reconstitute with 220 µl CYP2C9 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 CYP2C9 Assay Buffer and vortex thoroughly to yield a 100X stock solution of NADP+. Storeat -20°C, stable for at least 3 freeze/thaw cycles.CYP2C9 Substrate: Reconstitute with 55 µl anhydrous reagent-grade acetonitrile and vortex until fully dissolved to obtain a 10 mMstock 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 CYP2C9: Do not reconstitute until ready to use. Reconstitute with 230 µl CYP2C9 Assay Buffer and add 20 µl ofNADPH Generating System (100X). Mix thoroughly to ensure a homogenous solution (the solution will have a slightly opaque, milkyappearance), aliquot and store at -80°C. Avoid repeated freeze/thaw cycles and use aliquots within one month (the RecombinantHuman CYP2C9 will lose approximately 10% activity per week when stored at -80°C). Thaw aliquots rapidly at 37°C and place on iceuntil use (thawed aliquots should be used within 4 hours).

Kit Components

CYP2C9 Assay Buffer: 100 ml7-HFC Standard: 1 vialCYP2C9 Inhibitor (Tienilic Acid): 1 vialNADPH

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Generating System (100X): 1 vialβ-NADP+ Stock (100X): 1 vialCYP2C9 Substrate: 1 vialRecombinant Human CYP2C9: 1 vial

Detection method Fluorescence (Ex/Em 415/502 nm)

Compatible Sample Types

Human liver microsomes and liver S9 fractionsLysates of tissues and cultured cells, primary hepatocytesHeterologously expressed recombinant CYP2C9 preparations

Features & Benefits

• Simple, convenient, highly sensitive • Fluorescent assay enables easy determination of CYP2C9activity in a variety of biological samples • The substrate shows low background and a high signal-to-noise ratio • Kit includes CYP2C9 inhibitor (Tienilic Acid) and a stable, recombinant human CYP2C9 co-expressed with NADPH Reductase as a positive control

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