CREATIVE BIOMART®
Assay Kit

Cytochrome P450 2D6 Fluorometric Activity Assay Kit

Product Information

Cat.No. Kit-2134

Product Overview

CYP2D6 Activity Assay Kit enables rapid measurement of native or recombinant CYP2D6 activity in biological samples such as liver microsomes. The assay utilizes a non-fluorescent CYP2D6-selective substrate that is converted into a highly fluorescent metabolite detected in the visible range (Ex/Em = 390/468 nm), ensuring a high signal-to-background ratio with little interference by autofluorescence. CYP2D6 specific activity is calculated by running parallel reactions in the presence and absence of the potent CYP2D6-selective inhibitor quinidine and subtracting any residual activity detected with the inhibitor present. The kit contains a complete set of reagents sufficient for performing 100 sets of paired reactions (in the presence and absence of inhibitor).

Size

200 assays

Description

Cytochrome P450 2D6 (CYP2D6, 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. CYP2D6 catalyzes oxidation of lipophilic bases with an aromatic ring and a nitrogen atom and is highly expressed in liver and brain tissue. The enzyme is responsible for metabolism of nearly 25% of all small molecule drugs commonly used by humans, particularly psychiatric drugs such as antidepressants, antipsychotics and stimulants. The CYP2D6 gene is highly polymorphic in the human population, with CYP2D6 activity ranging from complete metabolic deficiency to ultra-rapid metabolism. Due to this wide phenotypic variability, CYP2D6 is frequently implicated in drug toxicity and clinical drug/drug interactions. In addition, for drugs whose pharmacological activity requires metabolism from a pro-drug form, CYP2D6 inhibition or allelic deficiency can lead to decreased drug efficacy.

Applications

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Rapid assessment of native/recombinant CYP2D6 activity in lysates or microsomal fractions prepared from tissues or cells. Screening of drugs and novel ligands for interaction with native/recombinant CYP2D6.

Target Species

Eukaryotes

Storage

Store kit at -20°C and protect from light. Briefly centrifuge all small vials prior to opening. Allow the CYP2D6 Assay Buffer to warm to room temperature prior to use. Read entire protocol before performing the assay procedure.AHMC Standard: Reconstitute in 110 µl of DMSO and vortex until fully dissolved to yield a 2 mM stock solution. The AHMC stock solution should be stored at -20°C and is stable for at least 3 freeze/thaw cycles.CYP2D6 Inhibitor (Quinidine): Reconstitute in 220 µl of acetonitrile and vortex until fully dissolved to yield a 2 mM stock solution. The stock solution is stable for 2 months at -20°C. To obtain a 15 µM working solution of quinidine (5X final concentration), add 15 µl of the 2 mM stock solution to 1985 µl of CYP2D6 Assay Buffer. Store the 15 µM quinidine solution at -20°C and use within one week.NADPH Generating System (100X): Reconstitute with 440 µl CYP2D6 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 440 µl CYP2D6 Assay Buffer and vortex thoroughly to yield a 100X stock. Store at -20°C, stable forat least 3 freeze/thaw cycles.CYP2D6 Substrate: Reconstitute with 220 µl anhydrous reagent-grade acetonitrile and vortex until fully dissolved. 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 CYP2D6: Do not reconstitute until ready to use. Reconstitute with 460 µl CYP2D6 Assay Buffer and add 40 µl of NADPH Generating System (100X). Mix thoroughly to ensure a homogenous solution, aliquot and store at -80°C. Avoid repeated freeze/thaw cycles and use aliquots within one month (the Recombinant Human CYP2D6 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

CYP2D6 Assay Buffer: 100 mlAHMC Standard: 1 vialCYP2D6 Inhibitor (Quinidine): 1 vialNADPH

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Generating System (100X): 1 vialβ-NADP+ Stock (100X): 1 vialCYP2D6 Substrate: 1 vialRecombinant Human CYP2D6: 1 vial

Detection method Fluorescence (Ex/Em 390/468 nm)

Compatible Sample Types

• Human liver microsomes and liver S9 fractions• Lysates of tissues and cultured cells, primary hepatocytes• Heterologously expressed recombinant CYP2D6 preparations

Features & Benefits

• Simple, convenient, highly sensitive • Fluorescent assay enables easy determination of CYP2D6 activity in a variety of biological samples The substrate shows low background and a high signal-to-noise ratio • Kit includes CYP2D6 inhibitor Quinidine and a stable, recombinant human CYP2D6 co-expressed with NADPH Reductase as a positive control

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