CREATIVE BIOMART®
Assay Kit

Cytochrome P450 1A2 Fluorometric Activity Assay Kit

Product Information

Cat.No. Kit-2126

Product Overview

CYP1A2 Activity Assay Kit enables rapid measurement of native or recombinant CYP1A2 activity in biological samples such as liver microsomes. The assay utilizes a non-fluorescent CYP1A2 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 selective CYP1A2 inhibitor is provided for determination of CYP1A2 activity in heterogeneous biological samples, where other CYP isozymes may contribute to substrate metabolism. The inhibitor displays greater than 20-fold selectivity for CYP1A2 over other CYPs, ensuring targeted inhibition. CYP1A2 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 1A2 (CYP1A2, 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. CYP1A2 is primarily expressed in liver, intestinal and olfactory mucosal tissue and catalyzes oxidation of polyaromatic and heterocyclic molecules such as aromatic amines. CYP1A2 is responsible for metabolism of approximately 10% of all small molecule drugs commonly used by humans. Polymorphisms in the human CYP1A2 gene have been implicated in clinical drug/drug interactions involving widely-used drugs, including caffeine, theophylline and the antipsychotic clozapine. Isoforms of the CYP1A subfamily are also involved in metabolic activation of environmental pro-carcinogens present in cigarette smoke and combustion exhaust fumes.

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Applications

Rapid assessment of native/recombinant CYP1A2 activity in lysates or microsomal fractions prepared from tissues, cells.Screening of drugs and novel ligands for interaction with native/recombinant CYP1A2.

Target Species

Eukaryotes

Storage

Store kit at -20°C and protect from light. Briefly centrifuge all small vials prior to opening. Allow the CYP1A2 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.CYP1A2 Inhibitor (a-naphthoflavone): Reconstitute in 110 µl of acetonitrile and vortex until fully dissolved to yield a 1 mM stock solution. The stock solution is stable for 2 months at -20°C. To obtain a 2.5 µM working solution of a-naphthoflavone (5X final concentration), add 5 µl of the 1 mM stock solution to 1995 µl of CYP1A2 Assay Buffer. The 2.5 µM working solution should be stored at -20°C and used within one week.NADPH Generating System (100X): Reconstitute with 220 µl CYP1A2 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 CYP1A2 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.CYP1A2 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 CYP1A2: Do not reconstitute until ready to use. Reconstitute with 230 µl CYP1A2 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 within one month (the Recombinant Human CYP1A2 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).

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Kit Components

CYP1A2 Assay Buffer: 100 ml3-CHC Standard: 1 viaCYP1A2 Inhibitor (a-naphthoflavone): 1 viaNADPH Generating System (100X): 1 viaβ-NADP+ Stock (100X): 1 viaCYP1A2 Substrate: 1 viaRecombinant Human CYP1A2: 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 CYP1A2 preparations

Features & Benefits

• Simple, convenient, highly sensitive • Fluorescent assay enables easy determination of CYP1A2 activity in a variety of biological samples • The substrate shows low background and a high signal-to-noise ratio • Kit includes CYP1A2 inhibitor (a-naphthoflavone) and a stable, recombinant human CYP1A2 co-expressed with NADPH Reductase as a positive control

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