CREATIVE BIOMART®
Assay Kit

Cytochrome P450 3A4 Inhibitor Screening Kit

Product Information

Cat.No. Kit-2142

Product Overview

CYP3A4 Inhibitor Screening Kit enables rapid screening of drugs and other new chemical entities (NCEs) for compound CYP3A4 interaction in a reliable, high-throughput fluorescence-based assay. The kit provides a yeast microsomal preparation of human CYP3A4 and cytochrome P450 reductase (CPR) enzymes. The assay utilizes a non-fluorescent CYP3A4 substrate that is converted into a highly fluorescent metabolite detected in the visible range (Ex/Em = 535/587 nm), ensuring a high signal-to-background ratio with little interference by autofluorescence. The concentration of fluorogenic substrate used for screening is roughly equivalent to its Km for CYP3A4, facilitating detection of weak competitive inhibitors. The kit contains a complete set of reagents sufficient for performing 200 reactions in a 96-well plate format.

Size

200 assays

Description

Cytochrome P450 3A4 (CYP3A4, EC 1.14.13.157) 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 transformed to more hydrophilic products to facilitate excretion from the body. CYP3A4 is expressed in high levels in the liver and intestines, where it catalyzes oxidation of an extraordinarily wide variety of structurally distinct ligands. More than half of all small molecule drugs commonly used by humans are metabolized by CYP3A4 and inhibition of CYP3A4-mediated metabolism is a common cause of adverse drug/drug and drug/food interactions and toxicity. In addition, for drugs whose pharmacological activity requires metabolism from a pro-drug form, CYP3A4 inhibition can lead to decreased drug efficacy.

Applications

Rapid, high-throughput screening of drugs and novel ligands. Development of structure-activity relationship (SAR) models to predict CYP3A4 inhibition liability of novel compounds.Prediction of

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adverse drug-drug interaction potential and bioavailability for compounds metabolized by CYP3A4.

Target Species

Eukaryotes

Storage

Store kit at -20°C and protected from light. Briefly centrifuge all small vials prior to opening. Allow the CYP3A4 Assay Buffer to warm to room temperature (RT) prior to use. Read entire protocol before performing the assay procedure.Resorufin Standard: Warm the Resorufin Standard to RT and vortex prior to use. The Resorufin Standard stock solution should be stored at -20°C and is stable for at least 3 freeze/thaw cycles.CYP3A4 Inhibitor (Ketoconazole): The inhibitor Ketoconazoleis provided as a dried film. Reconstitute in 220 µl of acetonitrile and vortex until fully dissolved to yield a 5 mM stock solution. The stock solution is stable for 2 months at -20°C. To obtain a 150 µM working solution of Ketoconazole (5X final concentration), add 30 µl of the 5 mM Ketoconazole stock solution to 970 µl of CYP3A4 Assay Buffer. Store the resulting 5X Ketoconazole working solution at -20°C and use within one week.NADPH Generating System (100X): Reconstitute with 220 µl CYP3A4 Assay Buffer, aliquot and store at -20°C. Avoid repeated freeze/thaw cycles. Keep on ice while in use.β-NADP+ Stock (100X): Dissolve in 220 µl CYP3A4 Assay Buffer and vortex thoroughly to yield a 10 mM stock solution of NADP+ (100X final concentration). Store at -20°C, stable for at least 3 freeze/thaw cycles.CYP3A4 Substrate: Reconstitute with 220 µl dry HPLC-grade acetonitrile and vortex until fully dissolved to obtain a 2 mM stocksolution. Store at -20°C. When using the CYP3A4 Substrate stock solution, allow the vial to warm to RT before opening and promptlyretighten cap after use to avoid absorption of airborne moisture.Recombinant Human CYP3A4: The Recombinant Human CYP3A4 should be reconstituted immediately before use as directed in Protocol.2 below. Each vial is sufficient for one 96-plate.

Kit Components

CYP3A4 Assay Buffer: 100 mlResorufin Standard (5 mM in DMSO): 50 μlCYP3A4 Inhibitor (Ketoconazole): 1 vialNADPH Generating System (100X): 1 vialβ-NADP+ Stock (100X): 1 vialCYP3A4 Substrate: 1 vialRecombinant Human CYP3A4: 2 vials

Detection method Fluorescence (Ex/Em 535/587 nm)

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Compatible Sample Types

Samples containing drugs, inhibitors or ligands (compounds that can interact and affect CYP3A activity)

Features & Benefits

• Simple, highly sensitive, high-throughput compatible • Rapid screening of CYP3A4 inhibitors or ligands • Kit includes the canonical CYP3A inhibitor ketoconazole and a stable, recombinant human CYP3A4 co-expressed with NADPH Reductase

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