CREATIVE BIOMART®
Assay Kit

Uridine diphospho-glucuronosyltransferases Activity Assay / Ligand Screening Kit

Product Information

Cat.No.

Kit-2109

Product Overview

UGT Activity Assay / Ligand Screening Kit enables rapid measurement of native or recombinant UGT activity in biological samples such as liver microsomes and can also be used to assess the effect of drugs and other novel compounds on UGT activity. The assay utilizes a highly fluorescent UGT substrate with a large Stokes shift (Ex/Em = 415/502 nm) that allows determination of UGT activity by tracking the drop in fluorescence emission as the substrate is converted into a non-fluorescent glucuronide. The multiisozyme substrate is glucuronidated by virtually all of the pharmacologically-relevant mammalian UGT1A and UGT2B enzymes. UGT specific activity is calculated by comparing the fluorescence loss versus a control reaction performed in the absence of the required cofactor UDPGA. The kit includes the pore-forming peptide antibiotic Alamethicin, which allows the UGT Substrate and UDPGA to rapidly diffuse across lipid membranes to access the UGT active site located in the lumen of microsomes. For verification of modulation of UGT activity by test ligands, diclofenac, a competitive inhibitor of most human and rodent UGT isozymes, is also included. The assay is highly sensitive, simple to perform and high-throughput adaptable. This assay can detect less than 0.1 mU UGT activity in biological samples. The kit contains a complete set of reagents sufficient for performing 100 reactions at a 100 μl reaction volume.

Size

100 assays

Description

Uridine diphospho-glucuronosyltransferases (UGTs, EC 2.4.1.17) are a superfamily of microsomal membrane-bound glycosyltransferase enzymes. UGTs are responsible for the vast majority of Phase II biotransformation (conjugation) reactions, in which a hydrophilic moiety is attached to small molecule drugs and other xenobiotics to facilitate their rapid excretion from the body. UGTs are predominantly expressed in the liver, intestine and kidneys, where they catalyze the addition of a glucuronic acid moiety from the nucleotide sugar uridine-5''-diphospho-a-D-glucuronic acid

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(UDPGA) to nucleophilic hydroxyl, sulfhydryl or amino groups of pharmacologically active drugs, Phase I drug metabolites and certain endogenous substrates such as steroid hormones, bile acids and bilirubin. Isozymes of the UGT family have extremely broad, often overlapping substrate specificities and tend to exhibit modest affinity for substrates but high catalytic turnover rates. UGTs also contribute to "first-pass" presystemic drug metabolism, which dramatically reduces the oral bioavailability of a number of drugs.

Applications

Rapid assessment of native/recombinant UGT activity in fractions prepared from tissues and cells.Screening of drugs and novel ligands for interaction with native/recombinant UGT enzymes.

Target Species

Mammalian

Storage

Store kit at -20°C and protect from light. Briefly centrifuge all small vials prior to opening. Allow the UGT Assay Buffer to warm to room temperature prior to use. Read entire protocol before performing the assay procedure.UGT Inhibitor (Diclofenac): Reconstitute in 550 µl of dH2O and vortex until fully dissolved to yield a 5 mM solution of diclofenac sodium. The solution should be stored at -20°C until use and is stable for at least 3 freeze/thaw cycles.UDPGA Stock (50X): Reconstitute with 220 µl dH2O to yield a 50X stock solution. Aliquot the stock solution as desired and store aliquots at -20°C. Avoid repeated freeze/thaw cycles and keep on ice while in use.Alamethicin: Alamethicin is provided as a solution in DMSO. Warm the solution to room temperature to melt the DMSO and vortex to ensure Alamethicin is completely dissolved. Aliquot and store at -20°C, stable for at least 3 freeze/thaw cycles.UGT Substrate (250X): Reconstitute with 110 µl reagent-grade DMSO and vortex until fully dissolved to obtain a 250X stock solution. The UGT Substrate should be stored at -20°C and is stable for at least 3 freeze/thaw cycles. Allow the vial to warm to room temperature before opening and promptly retighten cap after use to avoid absorption of airborne moisture.UGT Positive Control: Do not reconstitute until ready to use. Reconstitute with 22 µl UGT Assay Buffer and mix thoroughly to ensure a homogenous solution (the concentrated solution will be slightly viscous and have an opaque, milky appearance). The reconstituted UGT Positive Control may be aliquoted and stored at -80°C. Avoid repeated freeze/thaw cycles and use aliquots within one month. Thaw aliquots

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rapidly at 37°C and place on ice until use (thawed aliquots should be used within 4 hours).

Kit Components

UGT Assay Buffer: 100 mlUGT Inhibitor (Diclofenac): 1 vialUDPGA Stock (50X): 1 vialAlamethicin; 50 μlUGT Substrate (250X): 1 vialUGT Positive Control: 1 vial

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

Compatible Sample Types

Human or animal tissue microsomes and S9 fractions.Lysates of tissues and cultured cells (e.g. primary hepatocytes).Heterologously expressed recombinant UGT preparations

Features & Benefits

• Easy to use• Non-radioactive • Accurately measure UGT activity in microsomes, lysates and recombinant UGT preparations

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