CREATIVE BIOMART®
Assay Kit

Multidrug Efflux Transporter Ligand Screening Kit

Product Information

Cat.No. Kit-2094

Product Overview

The MDR1/P-gp Ligand Screening Kit is designed for rapidly screening test compounds for modulation of efflux transporter activity in MDR1-expressing cell lines. The assay uses a lipophilic non-fluorescent P-gp substrate that readily diffuses through the plasma membrane, where it is hydrolyzed to an active fluorophore by cytosolic esterases. The resulting hydrophilic fluorophore is neither membrane permeable nor a substrate for P-gp, hence it remains trapped inside the cell. In MDR1-expressing cell lines, the lipophilic pro-fluorophore is continuously extruded from the cytosol by P-gp, leading to a low intracellular fluorescence. Inhibition of P-gp-mediated efflux by a test compound leads to increased intracellular fluorescence. Specific transporter activity is quantified by comparison with fluorescence accumulated in the presence and absence of a saturating concentration of the included selective P-gp inhibitor. The assay is highly sensitive, has a simple no-wash protocol and is highthroughput adaptable. The kit contains a complete set of reagents sufficient for performing 100 reactions in a 96-well plate format.

Size

100 assays

Description

P-glycoprotein (P-gp, Multidrug Resistance Protein 1 (MDR1), EC 3.6.3.44) is a member of the ATP-binding cassette (ABC) ATPase superfamily of transmembrane transporter proteins. P-gp has an extremely broad substrate specificity and is capable of transporting a vast array of neutral and anionic lipophilic molecules. P-gp strongly affects the oral absorption, tissue distribution and excretion of many drugs and prevents certain lipophilic drugs from penetrating the blood brain barrier. Overexpression of P-gp confers tumor cells with resistance to chemically and pharmacologically distinct chemotherapeutic drugs (such as doxorubicin, vincristine and paclitaxel) by actively pumping them out of cells. Induction of P-gp expression is a frequent cause of treatment failure and tumor-targeted delivery of Pgp inhibitors is being investigated as a strategy for overcoming chemotherapy resistance.

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

Screening and characterization of drugs and new chemical entities for inhibition of native/recombinant MDR1/P-gp efflux transporter.Identification/characterization of cells and cell lines with a high level of MDR1-mediated efflux (i.e. chemotherapy-resistant).

Storage

Store kit at -20°C and protect from light. Briefly centrifuge all small vials prior to opening. Open all of the reagents under sterile conditions (e.g. a cell culture hood) only. Read entire protocol before performing the assay procedure.Efflux Assay Buffer: Allow to thaw to room temperature under sterile conditions. Store at 4°C.Fluorogenic P-gp Substrate: Reconstitute with 55 µl anhydrous DMSO and vortex thoroughly to obtain a 400X stock solution. Aliquot the stock solution as desired and store aliquots at -20°C, protected from light. Avoid repeated freeze/thaw cycles.P-gp Inhibitor (Verapamil): Reconstitute with 110 µl anhydrous DMSO and vortex until fully dissolved to obtain a 100X stock solution. Store at -20°C, stable for at least 4 freeze/thaw cycles.

Kit Components

Efflux Assay Buffer: 50 mlFluorogenic P-gp Substrate: 1 vialP-gp Inhibitor (Verapamil): 1 via

Detection method Fluorescence (Ex/Em = 488/532 nm)

Compatible Sample Types

Cells expressing high levels of MDR1/P-gp (e.g. cancer cell line with cytotoxic drug-resistant phenotype)

Features & Benefits

• Simple method to screen P-gp inhibitors• High-throughput adaptable• Includes Inhibitor Control, Verapamil

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