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DATA SHEET

MITOCHONDRIAL TOXICITY

Mitochondria perform two critical functions 1) the production of more than 90% of the cell's energy &, 2) control of cell survival as a part of programmed cell death (apoptosis). Assessing test compounds for mitochondrial toxicity is important in drug safety evaluation, with increasing focus early on in the development process.

Mitochondrial toxicants have little effect on cell growth or viability in glucose fed cells. Immortalised cell lines are metabolically adapted for rapid growth under hypoxic & acidic conditions, & derive almost all of their energy from glycolysis rather than via mitochondrial oxidative phosphorylation (OXPHOS), the Crabtree effect. In galactose fed cells, oxidation of galactose to pyruvate via glycolysis yields no net ATP, therefore cells are forced to rely on mitochondrial OXPHOS to generate sufficient ATP for survival. Replacing media glucose with galactose increases susceptibility of HepG2 Cells to mitochondrial toxicants.

In order to evaluate mitochondrial toxicity, two separate parallel cultures are grown 1) cells cultured as normal using glucose, & 2) cells cultured using galactose in absence of glucose as energy source.

Toxic effects of test compounds are evaluated in both cultures, where viabilities are compared for the assessment.

The assay provides an IG_{50} (concentration that causes inhibition growth of the 50% of the cell population) by means of a 7-point curve.
Deliverables: IG_{50} concentration of test compound in both cultures, & state if test compound is a mitochondria toxicant.

CUSTOMER PROVIDES

Compound identifier & molecular formula.

Test compound: 5mg solid

TEST COMPOUND REPLICATES

n=3 at each concentration (flexible).

FORMAT

96-well microplate, 100µL incubation volume, HepG2 cells.

PROTOCOL FOR PARALLEL CELL CULTURES (1) GLUCOSE-, & (2) GALACTOSE-, FED CELLS

Day 1: Cells at 80% confluency are diluted to 50,000 cells/mL in appropriate media, & 100µL are aliquoted into each well (5,000 cells/well). Plate is incubated to allow cells to attach overnight at 37°C and 5% CO2 humidified atmosphere.

Day 2: Test compound 10mM DMSO stocks are prepared & sterile filtered (0.2µm pore). Under sterile conditions, a test compound concentration range e.g. 0, 1, 5, 25, 50, 75, & 100µM is prepared in media such that the final DMSO concentration is 1% at each concentration (including the Zero test compound control [0µM] which will represent 100% viability).

Final Day: Media is removed to waste & replaced with detection reagent. After the detection reagent incubation time, well absorbances are measured by plate reader.

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CONTROLS

Rotenone is used as the standard quality control compound in each assay run.

Blank wells are prepared containing the solvent matrix: Media and 1% DMSO.

QUANTITATION

The 96-well plate is read on a UV/vis plate reader to measure absorbance, which is directly proportional to the number of viable cells.

DATA ANALYSIS AND RESULTS

The corrected absorbance value of the Zero (0µM test compound concentration) represents 100% viability. All wells containing test compound are compared with this well to calculate viabilities as follows:

$$\text{For each concentration of test compound, Viability} = \frac{\text{corrected Test absorbance}}{\text{corrected Zero absorbance}} \times 100\%$$

The % viability is plotted against test compound concentration, including zero, using curve fitting software, XLfit. The IG_{50} is determined from the graph (concentration giving 50% viability).

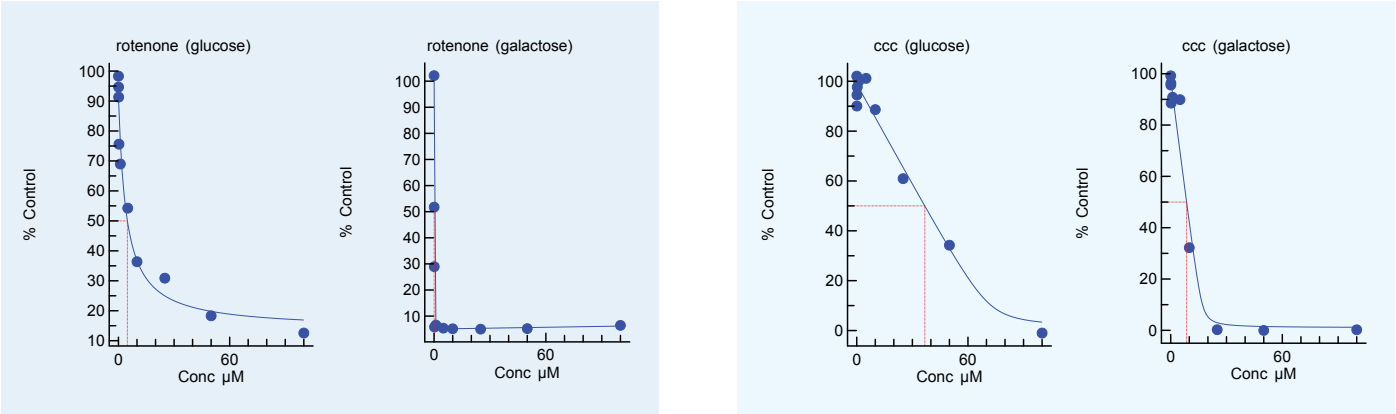

Figure1: Inhibition of HepG2 cell growth with known mitochondria toxic compounds rotenone & carbonyl cyanide 3-chlorophenylhydrazone (CCC); glucose & galactose fed cells directly compared. Viabilities are calculated with respect to Control cells grown under the same conditions in absence of rotenone or CCC.

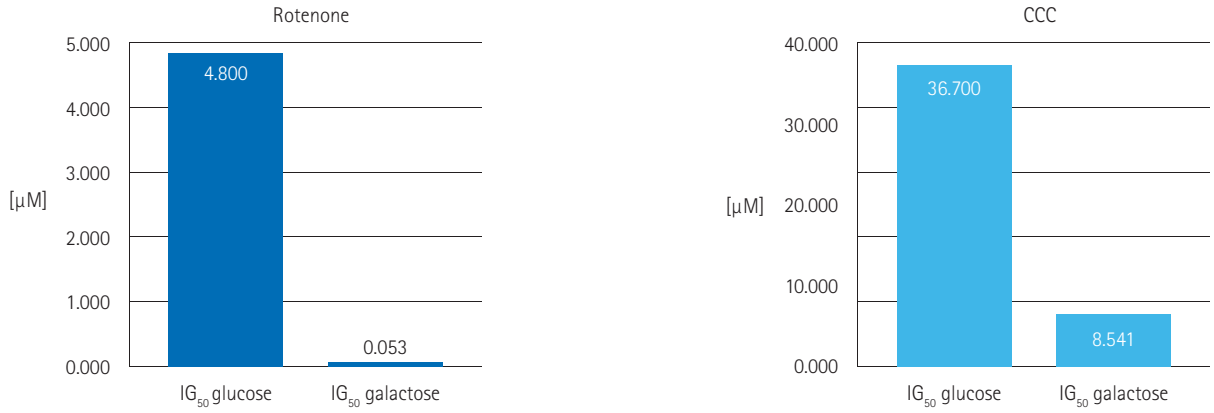

Figure2: IC50 results from figure 1 show increase in test article potency when cells fed with galactose rather than glucose. >1.5 fold difference in potency between growing conditions indicates mitochondria toxicity.

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