Application Note - Hepatotoxicity

Drug-induced mitochondrial toxicity measured in HepaRG® cells using the MitoXpress®-Xtra HS Oxygen Consumption Assay

Introduction

The choice of cell type is a critical parameter in the design of an in vitro toxicological assessment. For hepatotoxicity, the benchmark is primary human hepatocytes; however, such cells are associated with the obvious limitations of access, lot-to-lot variability and cost. Cell lines such as HepG2, THLE and Fa2N-4 are therefore commonly used as a cheaper more accessible alternative but these too are associated with significant limitations as they lack critical enzyme function such as CYP and transporter activity.

The HepaRG® cell type is an attractive alternative as it addresses a number of these limitations. It is a bi-potent hepatic progenitor cell line derived from an hepatocholangiocarcinoma (1) and differentiates into hepatocyte-like and biliary-like cells (2-3). The cells are received in cryopreserved format as a terminally differentiated co-culture and maintain many key primary human hepatocyte characteristics including CYP, MAO and specific transporter activity (4-5).

Here we describe how to assess the oxygen consumption of these cells using the MitoXpress®-Xtra HS product (MX-200) (Luxcel Biosciences) (6). Such analysis is conducted on a standard microtitre plate format and facilitates a detailed analysis of the mitochondrial function. These cells are an attractive model for the assessment of drug-induced mitochondrial dysfunction as they offer the opportunity to assess CYP and transporter mediated toxicity which is not possible with the standard alternative cell lines.

Fig 1: A) Phase contrast image of terminally differentiated after 15 days of culture and B) Immunofluorescence of HepaRG cells showing F- actin and CYP3A4 co-localisation.

Method

The differentiated HepaRG® cells (HPR116) are provided frozen in cryo vials at ≥ 8x10^6 viable cells per vial and are applied to collagen coated 96-well microtitre plates as per manufacturer’s instructions. Specific supplemented media is required for different aspects of culturing these cells which are provided, basal hepatic cell medium (MIL600) with either (i) the Thaw, Seed, and General Purpose supplement (ADD670) added or (ii) with Maintenance and Metabolism supplement (ADD620) added. These cells can be measured both in suspension immediately post thaw or as a monolayer either 4 hours or 4 days post thaw. The cells are prepared and added to the plate in the same way for both measurement types intended.

Plate Preparation

- Use the provided 96-well microtitre plates which are pre-coated with collagen I (PLA136).
- Thaw and count the viable differentiated HepaRG® cells as per manufacturer instructions, adjusting to ~4.8x10^5 cells/ml and adding to each well at 150µl per well.
Plate Measurement

Follow manufacturer’s protocol for probe preparation, addition and measurement. The following protocol is applicable for the measurement of cell suspensions or for cell monolayers 4 hours post seeding.

- Prepare a MitoXpress® stock in 1ml of pre-warmed Thaw, Seed, and General Purpose Medium 670
- Add 10μl of this solution to each well containing 150μl of cells and include a ‘no-cell’ control containing only media
- Add 1μl of compound stock (150X) to each well and ensure to include untreated samples
- Seal the plate by overlaying with pre-warmed HS mineral oil, 100μl per well. This is best done using a repeater pipette
- Measure 96-well plate kinetically for 90-120mins with ~2 minute interval (Ex/Em 380nm/650nm)

Contact Luxcel Biosciences on techsupport@luxcel.com if additional advice on instrument set-up is required.

Sample Data

Sample oxygen profiles are presented in Figure 2 for HepaRG® cells measured in suspension on a FLUOstar Omega plate reader (BMG Labtech) as outlined above. Untreated cells show a steady signal increase during measurement reflecting the depletion of oxygen caused by the activity of the electron transport chain (ETC).

When ETC activity is inhibited through treatment with complex III inhibitor Antimycin no signal increase is observed. When ETC is uncoupled via FCCP treatment a more rapid rate of signal change is observed.

Rates of signal change were calculated over the linear portion of the curve for each sample (~5-30mins), and can be used for the assessment of replicate statistics or the generation of dose response data. Antimycin A, Nefazadone dose responses are presented in Fig 3B and 3C.

The data illustrates the capacity of MitoXpress® to sensitively detect perturbed mitochondrial function in differentiated HepaRG® cells.

References

5. Guillouzo et al. (2007) CBI 168:66-73