PLATEFORME PUBLIC-PRIVÉ POUR LA PRÉ-VALIDATION DES MÉTHODES DE CARACTÉRISATION DES PERTURBATEURS ENDOCRINIENS
Pepper

In vitro assay for hepatic triglyceride accumulation

Liver steatosis is an accumulation of fat in liver that may lead to non-alcoholic fatty liver disease (NAFLD), and, in most serious cases, to a fibrosis of the liver that can induce major damage to the liver. Non-alcoholic fatty liver disease (NAFLD) is considered the most common liver disorder, affecting around 25% of the population worldwide. Currently, the essential role of environmental pollutants in NAFLD development is recognized. Particularly, endocrine-disrupting chemicals (EDCs) have a notable influence on this disease (Cano and al., 2021).

References

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Principle of the method

The ***in vitro* assay for hepatic triglyceride accumulation** measures the accumulation of triglycerides in human liver cells. This *in vitro* test method determines quantitatively the intracellular accumulation of triglycerides in HepaRG (human hepatocarcinoma) cells. Currently, HepaRG cells are the best known model to study human hepatocytes. This accumulation is measured using a fluorescent dye.

Differentiated HepaRG cells grown on a 96-well plate are treated for 72h (single dose) with the compound at eight different concentrations ranging up to the highest non-toxic concentration. A solvent- and a positive control are included on each plate.

Readouts : hepatic triglyceride accumulation, fluorescent dye

HepaRG cells are commercially available as cryopreserved non-differentiated cells from Biopredic International. Propagation, proliferation, and differentiation of the cells has to be performed in the laboratory where the test method is applied. HepaRG cells are passaged every two weeks. Experiments can be started using the third passage after thawing (after four weeks). Then cells are seeded directly on 96-well plates and kept in proliferation medium for two weeks, followed by differentiation medium for another two weeks. Once the cells are fully differentiated (four weeks after they have been seeded), one run is completed after 5 days. Hands on time is, however, minimal. Cells are cultivated in treatment medium for 48 h to adapt the cells to lower FCS and DMSO concentrations. Then incubation is performed for 72 h and the actual assay is performed within 15-20 minutes. Data analysis requires approximately 20 minutes.

Necessary equipment and consumables

- **Standard cell culture equipment** (e.g, incubator, laminar flow hood)
- **Material transfer agreement (MTA) for HepaRG HPR101 cultivation with Biopredict for academic scientists or a sub-license established between Biopredic International and researchers from commercial organisations**
- **Plate reader which can measure fluorescence** (96-well plate reader capable to measure AdipoRed at ex 485 nm/em 572 nm and Hoechst at ex 350 nm/em 461 nm)
- **96 wells-plate**