

***In vitro* assay for hepatic triglyceride accumulation**

The pre-validation is divided into two steps : a transferability and a second phase on blind-coded substances (30 substances).

A study plan and a report will be required by each lab for each phase.

The assay will be performed with **differentiated HepaRG - HPR116**.

Quantitative information

Transferability phase

- + **3 valid runs** (biological replicates) **per test item** are to be performed (a run is an independent experiment characterized by a new set of solutions and controls)
- + 2 substances per plate (several plates are performed in a day)
- + 1 full dose-response curve with positive control for each run
- + 1 positive control + **3 test items**
- + 8 concentrations per substance will be screened (in quadruplicates) (examples of plate layout in *figure 2*)
- + **Cytotoxicity** is measured for each plate

Phase II

- + **3 valid runs** (biological replicates) **per test item** are to be performed (a run is an independent experiment characterized by a new set of solutions and controls)
- + 2 substances per plate (several plates are performed in a day)
- + 1 full dose-response curve with positive control for each run
- + 1 positive control + **30 test items**
- + 8 concentrations per substance will be screened (in quadruplicates) (examples of plate layout in *figure 2*)
- + **Cytotoxicity** is measured for each plate

Quadruplicates

	1	2	3	4	5	6	7	8	9	10	11	12
A	PBS		C1	C2	C3	C4	C5	C6	C7	C8	Positive control	PBS
B	PBS											PBS
C	PBS	Solvent control (0.5 % DMSO)										PBS
D	PBS											PBS
E	PBS											PBS
F	PBS											PBS
G	PBS											PBS
H	PBS											PBS

Figure 2 : Example of a plate layout (C=concentration)

The offer should include:

- ✚ A contingency plan (including any changes about the workforce, work continuity during holidays...)
- ✚ A description of the quality system set up in the lab

The offer should specify:

- ✚ The quantity of needed HepaRG - HPR116 cells and corresponding media
- ✚ The associated cost