

## **Enzyme Induction Assay with Cultured Hepatocytes**

### **1. Objective**

Freshly plated hepatocytes represent a well established in vitro experimental model in which the induction of cytochrome P450 (CYP450) isoforms can be studied.

This assay evaluates the ability of test chemicals to induce CYP450 in cultured hepatocytes.

### **2. Introduction**

The cytochromes P450 are a superfamily of enzymes, which are found in all form of living organisms. In humans, they are drug metabolizing enzymes and hence are of great interest to pharmaceutical industries. Mutations in cytochrome P450 genes or deficiencies of the enzymes are also responsible for several human diseases. Some of these enzymes, are inducible, e. g. human CYP1A1, CYP2A6, CYP2B6, CYP2C9, CYP 2C19, CYP2E1, and CYP3A4. CYP450 induction is one of the key mechanisms for pharmacokinetic drug–drug interactions.

Freshly isolated human hepatocytes represent the most appropriate pre-clinical experimental system for the evaluation of CYP450 induction potential of xenobiotics in humans. Cryopreserved human hepatocytes have CYP450 activities and phase II conjugation enzymes activities similar to those of freshly isolated hepatocytes.

For more information please contact us!

CYP450 isoform	Inducer
1A2	Omeprazole
2A6 / 3A4	Rifampicin
2B6 / 2C19	Phenobarbital
2C9	Rifampicin / Phenobarbital

**Table 1: Typical inducers for CYP450**

### 3. Method

Cryopreserved hepatocytes are thawed and cultured in 24 well plates for 48 h.

After this recovery phase the media will be removed and replaced by the solutions of the test item, negative and positive controls. The hepatocytes will be incubated for 48 h or 72 h, whereas the medium has to be changed after 24 h.

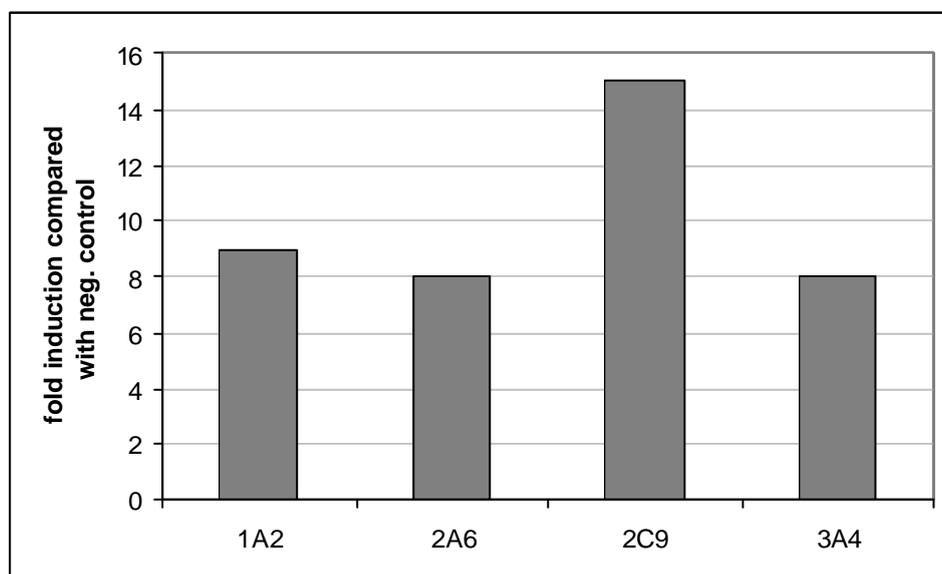
Afterwards the cells will be washed with a 3 mM salicylamid solution. Then a 3 mM salicylamid solution containing the respective probe substrate will be added and the 24 well plate will be further incubated for 1-3 hours. Afterwards the 3 mM salicylamid solution containing probe substrate will be harvested into suitable vials. The sample have to be analysed via HPLC, LC-MS or photometer to determine the extent of metabolism.

For more information please contact us!

CYP450 isoform	Substrate
1A2	Ethoxyresorufin
2A6	Coumarin
2B6	S-Mephenytoin
2C9	Diclofenac
2C19	S-Mephenytoin
2E1	Chlorzoxazone
3A4	Testosterone

**Table 2: CYP450 substrates**

## 4. Results



**Figure 1: Example of CYP450 induction with typical inducers**

Substances, which show a clear increase in CYP450 activities and/or a dose-effect relationship, have the ability to induce CYP450 in cultured hepatocytes.

For more information please contact us!