

Cloe Select Cytochrome P450 Induction

Background Information



'Cultured, primary human hepatocytes are the most accepted (industry, academia, regulatory) *in vitro* system for the potential for drug candidates to induce human P450 expression.'

PhRMA Perspective

Chu V, Einolf HJ, Evers R, Kumar G, Moore D, Ripp S, Silva J, Sinha V, Sinz M and Skerjanec A (2009) *Drug Metab Dispos* 37: 1339-1354

- Induction of cytochrome P450 enzymes is associated with an increased prevalence of clinical drug-drug interactions.
- Cloe Select Cytochrome P450 Induction assay identifies the potential of test compounds to induce CYP1A2, CYP2B6 or CYP3A4 in fresh cultured human hepatocytes by evaluating catalytic activity and mRNA levels as recommended in a recent PhRMA perspective (Chu *et al.*, 2009 *Drug Metab Dispos* 37:1339-1354).
- Test drug concentrations should be based on the expected human plasma drug concentrations. At least three concentrations spanning the therapeutic range should be studied, including at least one concentration that is an order of magnitude greater than the average expected plasma drug concentration. If this information is not available, concentrations ranging over at least two orders of magnitude should be studied.
- Cloe Select Cytochrome P450 Induction assay delivers data elucidating the extent of induction relative to positive and negative controls.
- The clinical consequences of induction may be therapeutic failure caused by a decreased systemic exposure of the drug itself or a co-administered therapy, or toxicity as a result of increased bioactivation.

Protocol

Test System

Fresh human hepatocytes

Test Compound Concentration

0.1 μ M, 1 μ M, 10 μ M (alternative concentrations available on request)

CYP Isoforms

CYP1A2, CYP2B6 and CYP3A4

Number of Replicates

3

Negative Control

Vehicle (typically 0.1 % DMSO)

Positive Control

Omeprazole (CYP1A2)
Phenobarbital (CYP2B6)
Dexamethasone and rifampicin (CYP3A4)

Compound Requirements

100 μ L of 10 mM solution

Exposure Period

72 hr (media changed every 24 hrs)

Probe Substrates

Ethoxyresorufin (CYP1A2)
Bupropion (CYP2B6)
Midazolam (CYP3A4)

Analysis method

Fluorescent quantification of resorufin (CYP1A2)
LC-MS/MS quantification of hydroxybupropion (CYP2B6) and 1-hydroxymidazolam (CYP3A4)
RT-PCR of mRNA expression (CYP1A2, CYP2B6 and CYP3A4).

Data Delivery

Report detailing methodology, donor demographics, concentration of metabolite of probe substrate (activity), mRNA levels, fold induction above vehicle control, probability (p value) of induction and % of positive control induction.

According to a recent PhRMA perspective (Chu *et al.*, 2009)¹, the most commonly used and recommended experimental protocol for assessing enzyme induction in regulatory submissions involves the use of primary hepatocytes and evaluates catalytic activity and mRNA levels for 3 cytochrome P450 isoforms, CYP1A2, CYP2B6 and CYP3A4.



Cloe Screen Cytochrome P450 Induction Validation

Induction of CYP1A2 by omeprazole, CYP2B6 by phenobarbital and CYP3A4 by rifampicin was investigated using cells from three individual human donors. All donors responded to the inducers, both in terms of catalytic activity and mRNA assessment.

Figure 1

Induction of CYP1A2 catalytic activity and mRNA levels by omeprazole (50 μ M) in human hepatocytes (Data represent mean fold induction of CYP1A2 \pm standard deviation for a single donor, n=3 replicates)

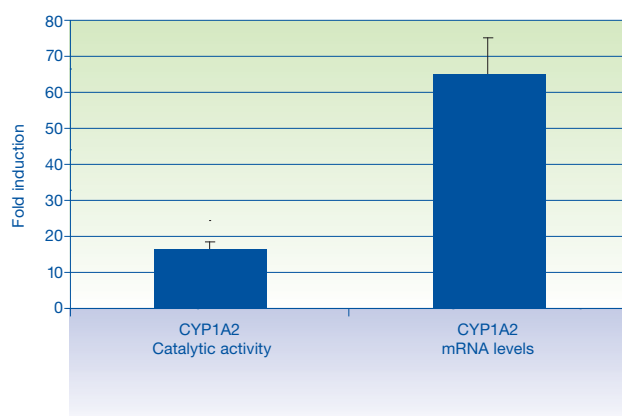


Figure 2

Induction of CYP2B6 catalytic activity and mRNA levels by phenobarbital (500 μ M) in human hepatocytes (Data represent mean fold induction of CYP2B6 \pm standard deviation for a single donor, n=3 replicates)

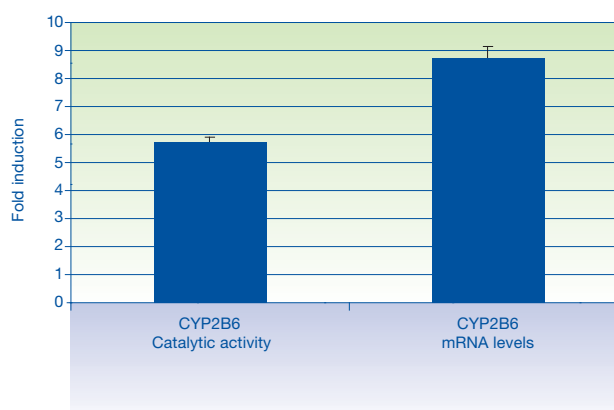
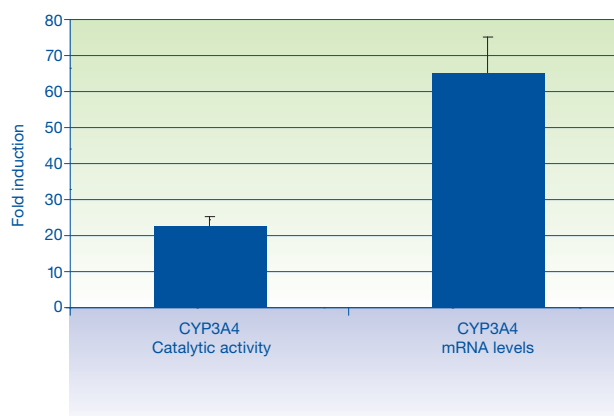


Figure 3

Induction of CYP3A4 catalytic activity and mRNA levels by rifampicin (10 μ M) in human hepatocytes (Data represent mean fold induction of CYP3A4 \pm standard deviation for a single donor, n=3 replicates)



References

¹ Chu V, Einolf HJ, Evers R, Kumar G, Moore D, Ripp S, Silva J, Sinha V, Sinz M and Skerjanec A (2009) *Drug Metab Dispos* **37**: 1339-1354