



## Study Design: CYP450 Induction using Human hepatocytes

### Test System

Cryopreserved human hepatocytes (3 donors recommended)

### Test Article Concentration

0.1, 1, 10  $\mu\text{M}$  (dependent upon unbound  $C_{\text{max}}$ , dose, solubility and cytotoxicity) plus vehicle control, in triplicate  
Or 6 concentrations (Calculate  $\text{EC}_{50}$ )

### CYP Isoforms

CYP1A2, CYP2B6 and CYP3A4

### Positive Control

Omeprazole (CYP1A2); Phenobarbital (CYP2B6); Rifampicin (CYP3A4)

### Test Article Requirements

Dependent on top concentration  
(recommend 0.1% DMSO of final concentration)

### Exposure Period

48-72 hr (Media changed every 24 hrs)

### Probe Substrates for Catalytic Activity

Phenacetin (CYP1A2)  
Bupropion (CYP2B6)  
Midazolam (CYP3A4)

### Analysis Method

LC-MS/MS quantification of acetaminophen (CYP1A2), hydroxybupropion (CYP2B6) and 1-hydroxymidazolam (CYP3A4)  
qRT-PCR for relative mRNA expression levels