A Bioluminescent Assay System for Measuring UDP Glucuronosyltransferase (UGT) Activity

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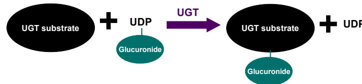
Abstract

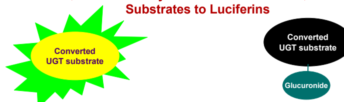
The UDP glucuronosyltransferase (UGT) family of enzymes are involved in the metabolism of various compounds in the body. These enzymes transfer a hydrophilic glucuronic acid moiety to their substrates, rendering them more water soluble and suitable for excretion. The UGTs act on various endogenous substrates, such as bilirubin, β-estradiol, and testosterone, as well as xenobiotics and drugs, such as diclofenac, morphine, and valproic acid. The function of these enzymes is essential for the clearance of drugs and other toxins from the body and alteration of UGT activity could potentially cause drug-drug interactions *in vivo*. There is an increasing interest in the activity of these enzymes and their involvement in drug metabolism. The current methods for assessing UGT enzyme activity are laborious and involve protein precipitation and/or chromatographic separation steps, which are not amenable to higher throughput screening applications for UGT inhibitors or activators. Here, we present a new bioluminescent assay system for measuring UGT enzyme activity in vitro. Our assay does not involve any protein precipitation or chromatographic steps and is easily performed in a multi-well plate format. We have shown the ability of our assay to measure activity of many recombinant UGT enzymes (including UGTs 1A1, 1A4, 1A6, 1A8, 1A9, 1A10, 2B7, and 2B15) as well as assessing endogenous UGT activities from animal tissue microsomes (including human liver, renal, and intestinal microsomes, as well as mouse and rat liver microsomes). We were able to detect inhibition by compounds known to inhibit numerous isozymes (diclofenac) and we verified published data showing that some isozymes are inhibited by the HIV protease inhibitors liponavir and ritonavir while other isozymes are relatively unaffected. Assay variability, as measured by Z' values, have been calculated for UGT 1A1 [Z' = 0.83] and UGT 2B7 [Z' = 0.67]. Our new assay format could greatly increase the throughput for assessing UGT activity and enable efficient screening of UGT isozymes against compound libraries.

UGT Reaction Scheme

Step 1: UGT Converts Substrate to a Glucuronide



Step 2: Luciferin Detection Reagent plus D-Cysteine Converts Substrates to Luciferins



Conversion of original substrate gives light producing luciferin but the glucuronide does not give light.

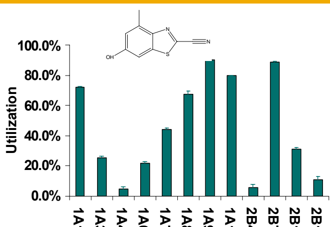
- UGT activity results in a drop of light output from the starting point
- Plotting the difference between the starting point and the experimental points allows visualization of the data in a more traditional way (ie., UGT activity shows a positive signal)

Reaction Conditions

- 50 mM TES buffer, pH 7.5
- 5 mM $MgCl_2$
- 4-5 mM UDPGA
- 25 µg/ml alamethicin
- 10-200 µM substrate (depending on isozyme(s))
- 0.05 – 0.3 mg/ml UGT enzyme (isozyme dependent)
  - UGT Supersomes™ (BD Gentest)
  - Tissue microsomes (Xenotech or BD Gentest)

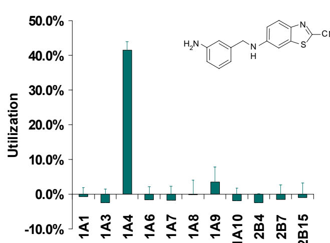
- UGT reactions are run at 37°C.
- Reaction time for Supersomes™ is 30 min to 3 hours.
- Reaction time for tissue microsomes is 10 min – 2 hrs.

Substrate Specificity of UGT Substrate #1



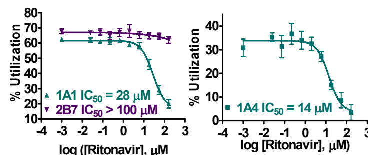
Supersomes™ (0.2 mg/mL) were screened against substrate #1 (30 µM final) in quadruplicate in a 96-well assay plate (40 µL reactions) at 37°C for 2 hrs.
The compound utilization was calculated by subtracting the +UDPGA sample from the –UDPGA sample and dividing that value by the original starting –UDPGA value.

Substrate Specificity of UGT Substrate #2



Reactions were performed the same as substrate #1, except substrate #2 was 25 µM final.

Effect of Ritonavir on UGT Supersomes™

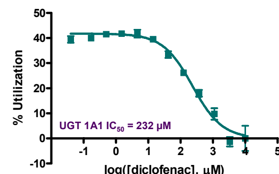


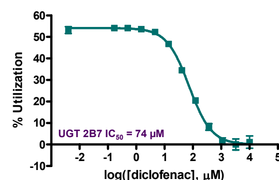
UGT inhibition assays were carried out for 1-2 hours at 37°C under standard reaction conditions with increasing amounts of the HIV protease inhibitor ritonavir. Supersomes™ were present in the reaction at 0.2 mg/ml. 1A1 and 2B7 Supersomes were assayed with 50 µM substrate #1 and 1A4 Supersomes were assayed with 25 µM substrate #2.

Literature reports[1] an IC_{50} value of 2.0 µM for 1A4 using trifluoperazine as the substrate, and IC_{50} value of 19.0 µM for 1A1 using bilirubin as the substrate and no significant inhibition of 2B7 (IC_{50} > 100 µM) using 7-hydroxy trifluoromethyl coumarin as the substrate.

[1]Zhang *et. al. Drug Metab. Disp.* (2005). 33:11, 1729-1739.

Effect of Diclofenac on UGT Supersomes™



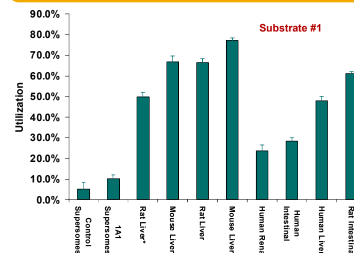


UGT inhibition assays were carried out for 90 min at 37°C under standard reaction conditions with increasing amounts of the diclofenac sodium. Supersomes™ were present in the reaction at 0.1 mg/ml and compound #1 was present at 20 µM, which is the approximate S_{50} value for UGT 1A1 and 2B7.

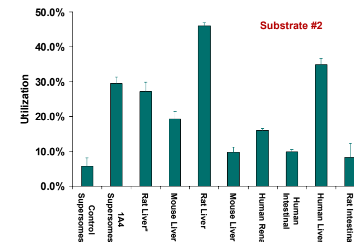
Results are consistent with literature reports that UGT 2B7 is inhibited more potently than UGT 1A1 by diclofenac using 4-methylumbelliferone as the substrate[1].

[1]Uchaipichat *et al. Drug Metab. Disp.* (2004). 32:4, 413-423.

UGT Activity with Tissue Microsomes

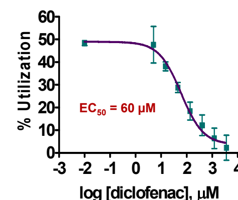


Tissue microsomes (0.1 mg/mL) and Supersomes™ (0.2 mg/mL) were screened against substrate #1 (50 µM final) in quadruplicate in a 96-well assay plate (40 µL reactions) at 37°C for 15 minutes.
Microsomes with * from BD Gentest. All others purchased from Xenotech LLC.



Tissue microsomes (0.2 mg/mL) and Supersomes™ (0.2 mg/mL) were screened against substrate #2 (50 µM final) in quadruplicate in a 96-well assay plate (40 µL reactions) at 37°C for 2 hrs.
Microsomes with * from BD Gentest. All others purchased from Xenotech LLC.

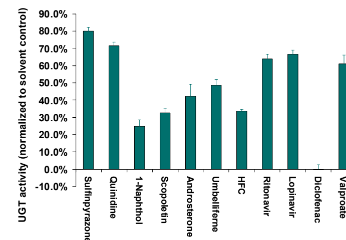
Inhibition of Human Liver Microsome UGT by Diclofenac



Human Liver Microsomes (0.1 mg/ml) were incubated for 15 min at 37°C with 50 µM substrate #1 and increasing concentrations of diclofenac. Points were determined in quadruplicate in a 96-well assay plate. Diclofenac has been shown to inhibit most of the UGT isozymes to varying degrees[1].

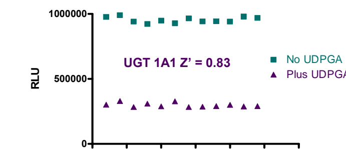
[1]Uchaipichat *et al. Drug Metab. Disp.* (2004). 32:4, 413-423.

Effect of Various UGT Inhibitors on UGT Activity in Human Liver Microsomes



Human liver microsome (HLM) UGT inhibition assays were carried out for 15 min at 37°C under standard reaction conditions using 0.05 mg/ml HLM. Substrate #1 was used at 50 µM. Ritonavir and lopinavir were used at 0.25 mM. Sulfinpyrazone, quinidine, and androsterone were used at 0.5 mM. 1-Naphthol, scopoletin, umbelliferone, and 7-hydroxy-4-trifluoromethyl coumarin (HFC) were used at 1 mM. Diclofenac was used at 5 mM. Valproate was used at 10 mM.

Assay Variability



Z' value for recombinant UGT 1A1 Supersomes™ (0.2 mg/ml) determined under standard assay conditions after a 2 hour reaction at 37 °C.
Z' value for recombinant UGT 2B7 Supersomes™ was determined to be 0.67 under the same conditions.

Conclusions

- This assay can be used to detect the activity of many of the UGT isozymes in a multiwell format
- This assay can be used with recombinant UGT enzymes or tissue microsomes (ie, human liver)
- The small reaction size (40 µL in a 96 well plate) saves the researcher money on costly microsomal enzyme preps, UDPGA, and test compounds
- Unlike conventional UGT assay methods, this assay lacks protein precipitation, centrifugation, and chromatography steps, making it amenable to HTS