Promega

Technical Bulletin

UGT-Glo™ Assay

INSTRUCTIONS FOR USE OF PRODUCTS V2120, V2121, V2130, V2131, V2081 AND V2082.

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UGT-Glo™ Assay

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1. Description

The UGT-Glo™ Assay[a,b] provides a luminescent method for measuring UDP glucuronosyltransferase (UGT) activity. The 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 UGT-Glo™ Assay is designed to measure UGT activity from a variety of sources, such as microsomes containing recombinantly expressed enzymes or microsomal preparations derived from mammalian tissues, and to test the effect of various chemicals on UGT activity. The assay uses proluciferin substrates for UGT enzymes (Figure 1). The specificity of the substrates for a panel of recombinant UGT isozymes is shown in Figure 2. These substrates are first modified by UGT enzymes and then converted to the luciferin derivatives using D-Cysteine during the detection step (Figure 3). Without conversion to a luciferin derivative by D-Cysteine, the proluciferin substrates will not produce light when incubated with luciferase.

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1. Description (continued)

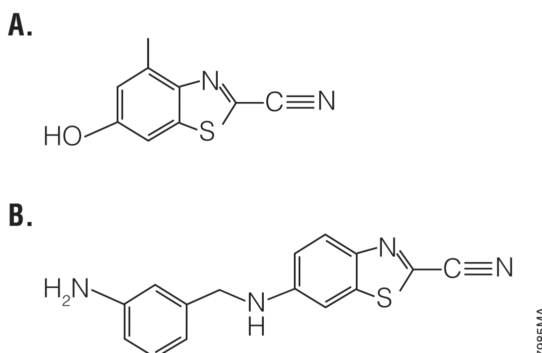

Figure 1. Proluciferin UGT substrates. The UGT Multienzyme Substrate (**Panel A**) is glucuronidated by many recombinant UGT enzymes, including UGT1A1, UGT2B7, UGT1A8, UGT1A9, UGT1A10 and UGT2B15. The UGT1A4 substrate (**Panel B**) is glucuronidated by recombinant UGT1A4 and, to a lesser extent, recombinant UGT1A9.

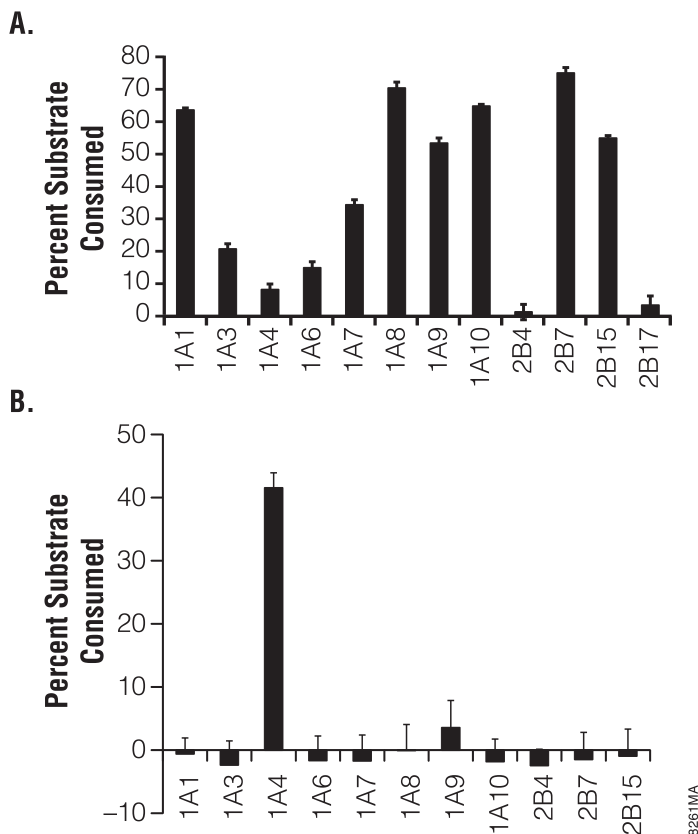

Figure 2. Specificity of UGT substrates for a panel of UGT isozymes. Panel A. Forty microliter UGT-Glo™ Assays were performed with 30µM UGT Multienzyme Substrate and 0.2 mg/ml of various UGT Supersomes™ (BD Gentest). Reactions were incubated at 37°C for 2 hours. **Panel B.** Experiment performed as in Panel A except for using 25µM UGT1A4 Substrate. The numbers are background subtracted.

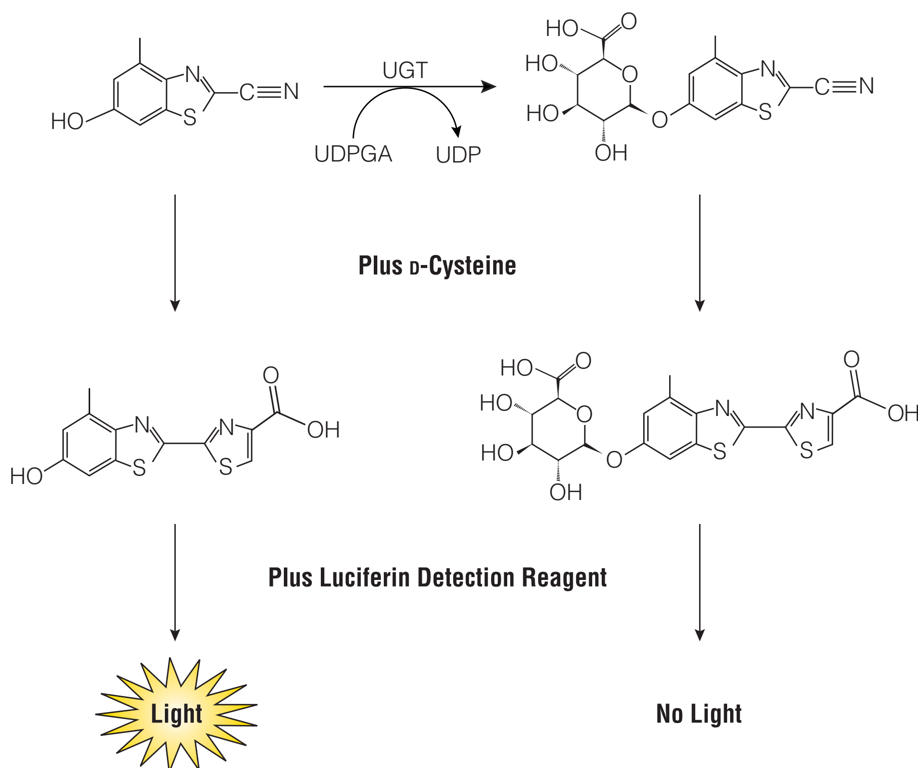

Figure 3. Conversion of UGT Multienzyme Substrate by UGT enzymes. UGT enzymes attach a glucuronic acid moiety to the proluciferin substrate. During the detection step, the proluciferin is simultaneously converted to a luciferin by cyclization with D-Cysteine. Luciferase uses the luciferin analog of the initial substrate to produce light but does not produce light with the glucuronidated luciferin. Light output is inversely proportional to UGT enzymatic activity.

To measure UGT activity with the UGT-Glo™ Assay, two glucuronidation reactions are set up in parallel. Both reactions contain a source of UGT and the proluciferin substrate, but only one of them contains the uridine 5´-diphosphoglucuronic acid (UDPGA) cofactor. During the incubation period with the UGT enzyme or enzymes, a portion of the proluciferin substrate is glucuronidated in the reaction containing UDPGA. None of the proluciferin is glucuronidated in the reaction lacking UDPGA. In the second step of the assay, incubation of the proluciferin substrates with D-Cysteine in the Luciferin Detection Reagent results in conversion of the proluciferins into luciferin molecules (Figure 3). Luciferin produced from the unmodified proluciferin will give light in the Luciferin Detection Reagent, but the luciferin produced from the glucuronidated proluciferin will not give light. Therefore, the decrease in light output when comparing the plus-UDPGA reaction to the minus-UDPGA reaction is proportional to the glucuronidation activity in the first step.

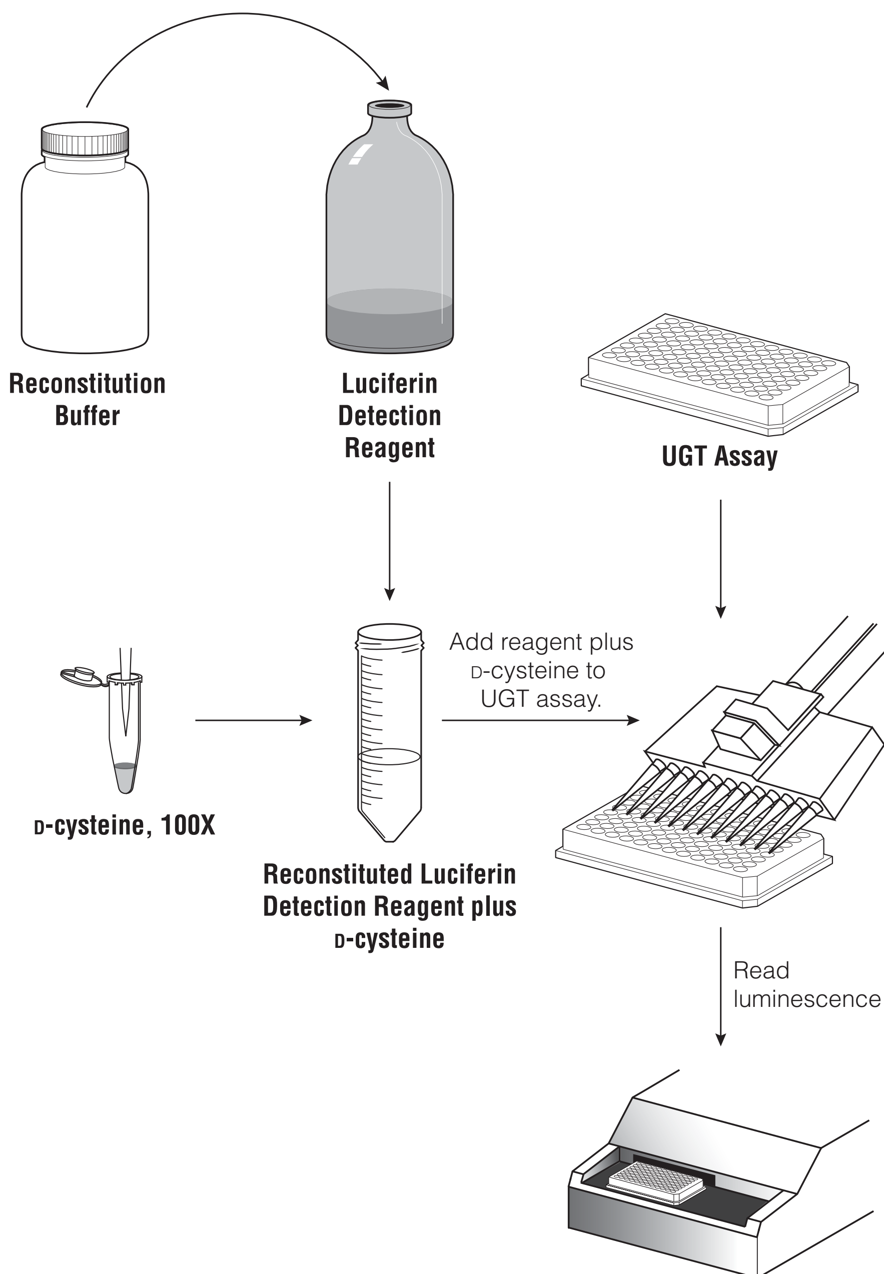

Figure 4. Flow diagram showing preparation and use of the reconstituted Luciferin Detection Reagent plus D-Cysteine. The entire contents of the Reconstitution Buffer are transferred into the amber Luciferin Detection Reagent bottle and gently mixed until homogeneous. The desired amount of reconstituted Luciferase Detection Reagent is dispensed to a separate tube, and D-Cysteine is added to a final concentration of 1X. Reconstituted Detection Reagent without added D-Cysteine can be stored at –20°C for later use.

Advantages of the UGT-Glo™ Assay System include:

Speed: The luminescent format can be completed in under 2 hours; eliminates the need for time-consuming analyses such as HPLC or LC/MS.

Simplified Method: The simple "add and read" protocol makes the assay amenable to high-throughput screening.

No Fluorescence Interference: The UGT-Glo™ Assay System eliminates fluorescence interference by using luminescence to monitor enzyme activity. In luminescent assays, there is no concern about possible overlap between the fluorescent excitation and emission wavelengths of reagents and test compounds. Such overlaps in fluorescent assays confound analysis and present misleading or irrelevant data.

Low False-Positive Rate: Use of a proprietary stabilized firefly luciferase (Ultra-Glo™ Recombinant Luciferase) and a proprietary luciferase buffer formulation minimizes the incidence of false positives due to luciferase inhibition.

Signal Stability: Glow-type luminescence provides a stable signal with a half-life of greater than 2 hours.

2. Product Components and Storage Conditions

Product	Size	Cat.#
UGT-Glo™ Assay	200 assays	V2081

This system contains sufficient reagents for 200 assays at 40µl per assay in 96-well plates or 400 assays at 20µl per assay in low-volume 384-well plate. Includes:

- 10µl UGT Multienzyme Substrate, 50mM
- 10µl UGT1A4 Substrate, 50mM
- 10ml Reconstitution Buffer
- 1 vial Luciferin Detection Reagent
- 1.6ml UGT Buffer, 5X
- 640µl UDPGA, 50mM
- 100µl D-Cysteine, 100X

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2. Product Components and Storage Conditions (continued)

Product	Size	Cat.#
UGT-Glo™ Assay	1,000 assays	V2082

This system contains sufficient reagents for 1,000 assays at 40µl per assay in 96-well plates or 2,000 assays at 20µl per assay in low-volume 384-well plate. Includes:

- 50µl UGT Multienzyme Substrate, 50mM
- 50µl UGT1A4 Substrate, 50mM
- 50ml Reconstitution Buffer
- 1 vial Luciferin Detection Reagent
- 8ml UGT Buffer, 5X
- 3.2ml UDPGA, 50mM
- 500µl D-Cysteine, 100X

Product	Size	Cat.#
UGT-Glo™ UGT1A1 Screening System	200 assays	V2120

Composed of V2081 and V2411. This system contains sufficient reagents for 200 assays at 40µl per assay in 96-well plates or 400 assays at 20µl per assay in low-volume 384-well plate. Includes:

- 10µl UGT Multienzyme Substrate, 50mM
- 10µl UGT1A4 Substrate, 50mM
- 10ml Reconstitution Buffer
- 1 vial Luciferin Detection Reagent
- 1.6ml UGT Buffer, 5X
- 640µl UDPGA, 50mM
- 100µl D-Cysteine, 100X
- 160µl UGT1A1 Microsomes (5mg/ml)
- 100µl Control Membranes

Product	Size	Cat.#
UGT-Glo™ UGT1A1 Screening System	1,000 assays	V2121

Composed of V2082 and V2412. This system contains sufficient reagents for 1,000 assays at 40µl per assay in 96-well plates or 2,000 assays at 20µl per assay in low-volume 384-well plate. Includes:

- 50µl UGT Multienzyme Substrate, 50mM
- 50µl UGT1A4 Substrate, 50mM
- 50ml Reconstitution Buffer
- 1 vial Luciferin Detection Reagent
- 8ml UGT Buffer, 5X
- 3.2ml UDPGA, 50mM
- 500µl D-Cysteine, 100X
- 800µl UGT1A1 Microsomes (5mg/ml)
- 100µl Control Membranes

Product	Size	Cat.#
UGT-Glo™ UGT2B7 Screening System	200 assays	V2130

Composed of V2081 and V2451. This system contains sufficient reagents for 200 assays at 40µl per assay in 96-well plates or 400 assays at 20µl per assay in low-volume 384-well plate. Includes:

- 10µl UGT Multienzyme Substrate, 50mM
- 10µl UGT1A4 Substrate, 50mM
- 10ml Reconstitution Buffer
- 1 vial Luciferin Detection Reagent
- 1.6ml UGT Buffer, 5X
- 640µl UDPGA, 50mM
- 100µl D-Cysteine, 100X
- 160µl UGT2B7 Microsomes (5mg/ml)
- 100µl Control Membranes

Product	Size	Cat.#
UGT-Glo™ UGT2B7 Screening System	1,000 assays	V2131

Composed of V2082 and V2452. This system contains sufficient reagents for 1,000 assays at 40µl per assay in 96-well plates or 2,000 assays at 20µl per assay in low-volume 384-well plate. Includes:

- 50µl UGT Multienzyme Substrate, 50mM
- 50µl UGT1A4 Substrate, 50mM
- 50ml Reconstitution Buffer
- 1 vial Luciferin Detection Reagent
- 8ml UGT Buffer, 5X
- 3.2ml UDPGA, 50mM
- 500µl D-Cysteine, 100X
- 800µl UGT2B7 Microsomes (5mg/ml)
- 100µl Control Membranes

Storage Conditions: Avoid multiple freeze-thaw cycles of all components. Store the UGT1A1 and UGT2B7 Microsomes and Control Membranes at –70°C. UGT enzymes may lose activity with repeated freeze-thaw cycles. Avoid multiple freeze-thaw cycles by dispensing the UGT1A1 and UGT2B7 membranes into single-use aliquots (e.g., 80µl per 96 reactions). Store aliquots at –70°C. Store other components at –20°C. Protect components from light.

The reconstituted Luciferin Detection Reagent **without D-Cysteine added** can be stored at –20°C for up to three months. For convenience, the reconstituted Luciferin Detection Reagent **without D-Cysteine added** can be stored at room temperature (approximately 23°C) for 24 hours or at 4°C for 1 week without loss of activity.

Use reconstituted Luciferin Detection Reagent plus D-Cysteine the same day. Do **not** store reconstituted Luciferin Detection Reagent plus D-Cysteine.

3. Performing the UGT-Glo™ Assay

3.A. General Information

The UGT-Glo™ Assays are performed in two steps (Figure 3).

Step 1. The UGT Reaction: The first step of the UGT-Glo™ Assay combines the UGT substrate with UGT enzyme and UDPGA to generate a glucuronidated product. Under standard conditions, the resultant mixture contains both glucuronidated proluciferin and unmodified proluciferin. The recommended final substrate concentrations, enzyme concentrations and incubation time, as well as the apparent S_{50} values for the substrates, are shown in Table 1. The S_{50} value is similar to the K_m value derived from the Michaelis-Menten equation but is derived using the Hill equation, which more accurately fits UGT enzyme kinetics. The magnitude of the UGT-Glo™ Assay signal will vary with the specific activity of the UGT preparation. Optimization of the amount of enzyme or time of incubation may be required for new preparations of enzyme. UGT-Glo™ Assays are performed in UGT Buffer.

Note: All data shown in this Technical Bulletin are from experiments performed in 1X UGT Buffer plus 25µg/ml alamethicin. Alamethicin is a mixture of peptides from a bacterial source that forms pores in cellular membranes and is used to allow molecules to pass into cells or microsomes that otherwise may not diffuse. Studies of inhibition with UGT2B7 give the same IC_{50} values with and without alamethicin although the starting amount of substrate consumed is higher with alamethicin present.

Step 2. The Luciferin Detection Reaction: The proluciferin mixture present after glucuronidation in Step 1 reacts with the D-Cysteine added to the Luciferin Detection Reagent to form luciferin analogs of the proluciferin species. Both the glucuronidated and the unmodified proluciferin are converted to luciferin analogs. The other components of the Luciferin Detection Reagent react with the unmodified luciferin analog to give light; however, the glucuronidated luciferin product is not a substrate for luciferase and therefore does not generate light. The UGT-Glo™ Assays use a proprietary luciferase (Ultra-Glo™ Recombinant Luciferase) to generate a stable "glow-type" luminescent signal. This eliminates the need for strictly timed luminescence detection.

Table 1. Recommended Assay Conditions for Recombinant UGT Enzymes.

UGT Enzymes	S_{50} (µM)	Substrate Type	Substrate Concentration (µM)	Time (Minutes)	Enzyme Concentration (mg/ml)
UGT1A1	20	Multienzyme	20	90	0.1
UGT2B7	20	Multienzyme	20	45–60	0.1
UGT1A8	125	Multienzyme	50	60	0.1–0.2
UGT1A9	1	Multienzyme	20	60	0.1
UGT1A10	50	Multienzyme	50	60	0.2
UGT2B15	20	Multienzyme	20	30	0.4
UGT1A4	70	1A4	50	120	0.4
UGT1A9	10	1A4	50	180+	0.4+

3.B. Assay Time and Enzyme Concentration

UGT reactions are generally performed at 37°C. The linear range for many UGT isozymes has been determined at several enzyme concentrations in 1X UGT buffer plus 25µg/ml alamethicin (Table 2, Figures 5 and 6). Table 2 gives the approximate time (in minutes) over which the reaction was approximately linear for the batches of Supersomes™ (BD Gentest) used. In general, most of the UGT reactions are linear with respect to time until they reach approximately 40–50% consumption of the substrate (Figures 5 and 6). After this point, the rate of reaction slows because enough substrate has been consumed to change the rate of the reaction.

Note: UGT enzyme preparations are normally standardized to total protein content and not UGT-specific activity. The guidelines given here can be used as a starting point, but the linear range of each new batch or lot of enzyme should be experimentally verified. Running inhibition assays starting at more than 50% utilization of the substrate frequently leads to overestimation of IC_{50}.

3.B. Assay Time and Enzyme Concentration (continued)

Table 2. Linearity of UGT Reactions Over Time At Various Enzyme Concentrations.

UGT Enzymes	Substrate Type	Substrate (µM)	Incubation Time for 0.05mg/ml UGT Enzyme (minutes)	Incubation Time for 0.1mg/ml UGT Enzyme (minutes)	Incubation Time for 0.2mg/ml UGT Enzyme (minutes)	Incubation Time for 0.4mg/ml UGT Enzyme (minutes)
UGT1A1	Multienzyme	20	150	90	60	nd
UGT2B7	Multienzyme	20	150	90	45	nd
UGT1A8	Multienzyme	50	150	90	60	nd
UGT1A9	Multienzyme	20	150	90	45	<30
UGT1A10	Multienzyme	50	120	120	60	nd
UGT2B15	Multienzyme	20	nr	90	45	30
UGT1A4	1A4	50	nr	210	120	120
UGT1A9	1A4	50	nr	nr	210	180
nd = not determined; nr = not recommended						

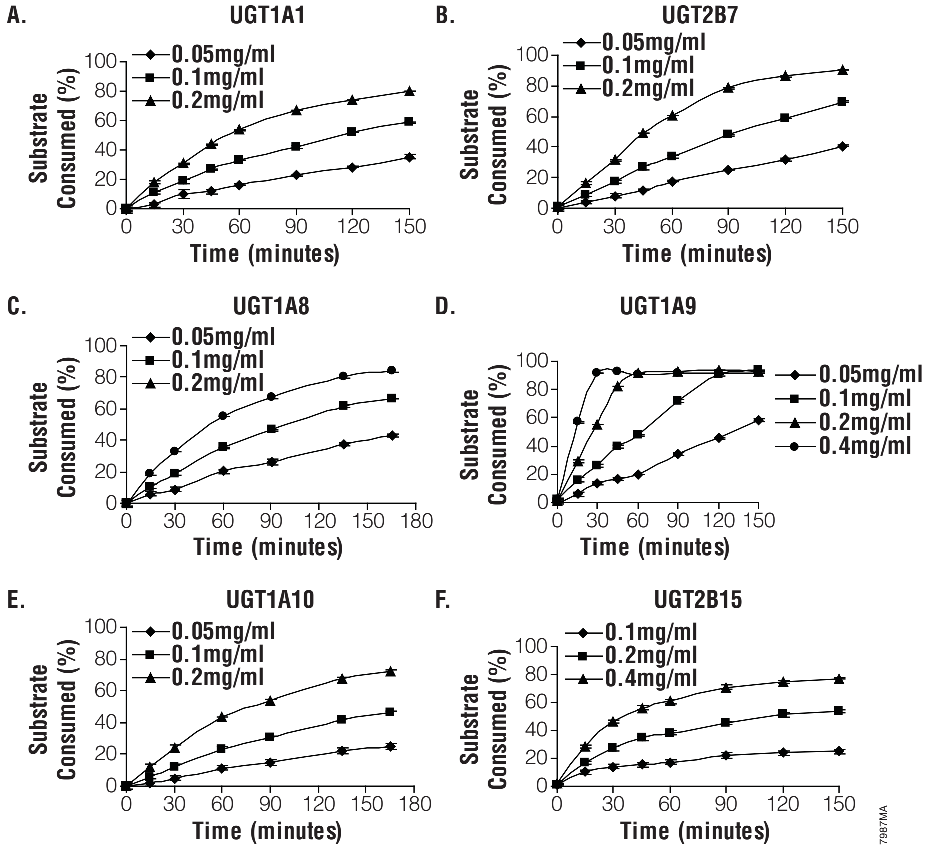

Figure 5. Incubation time and enzyme concentration for Multienzyme Substrate. UGT-Glo™ reactions (40µl) were performed with the recommended Multienzyme Substrate concentrations indicated in Table 1 at several enzyme concentrations. Luminescence was measured using a GloMax® 96 Microplate Luminometer (Cat.# E6501), and the amount of substrate consumed was plotted (see Section 6 for the calculations).

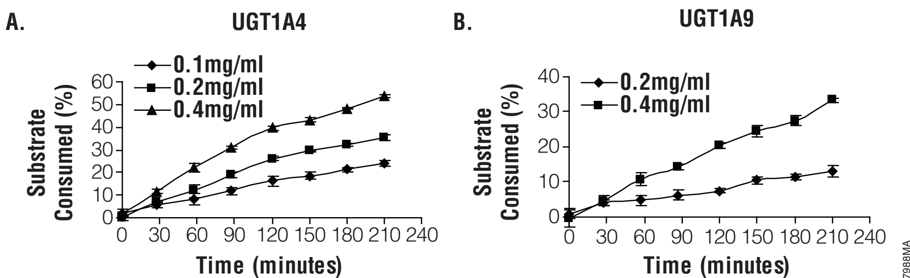

Figure 6. Incubation time and enzyme concentration for UGT1A4 Substrate. UGT-Glo™ reactions (40µl) were performed with the recommended UGT1A4 substrate concentrations indicated in Table 1 at several enzyme concentrations. Luminescence was measured using a GloMax® 96 Microplate Luminometer (Cat.# E6501) and the amount of substrate consumed was plotted (see Section 6 for the calculations).

4. General Overview of Protocol

Step 1

1. Add 10µl of 4X test compounds to each well of a white opaque 96-well plate.
2. Add 10µl of 16mM UDPGA to each plus-UDPGA well and 10µl of water to each minus-UDPGA well.
3. To start the reaction, add 20µl of a mixture containing 2X UGT-Glo™ Buffer, 2X UGT substrate and 2X enzyme to each well.
4. Incubate at 37°C for the appropriate amount of time (see Table 2).

Step 2

1. Add 40µl of reconstituted Luciferin Detection Reagent plus D-Cysteine to each well.
2. Allow the luminescent signal to stabilize at room temperature for 20 minutes.
3. Read luminescence.

Materials to Be Supplied by the User
(Solution compositions are provided in Section 7.A.)

- white opaque multiwell plates (e.g., 96-well Costar® plates, Corning Cat.# 3912). Do **not** use treated plates, clear plates or black plates.
- alamethicin (optional; Sigma Cat.# A4665). All data in this Technical Bulletin uses alamethicin in the UGT reaction at a final concentration of 25µg/ml. Add alamethicin (20mg/ml stock diluted in methanol) to the UGT Buffer, 5X, to a final concentration of 125µg/ml.
- luminometer or charge-coupled device (CCD) capable of reading multiwell plates. (Operate a multifunctional instrument in luminescence mode only.)
- plate sealers for water bath incubations [e.g., Phenix Research Products SealPlate adhesive sealing films (Cat.# LMT-THIN-EXS); optional]
- multichannel pipette or automated pipetting station for reagent delivery (optional)
- multiwell plate shaker for mixing plates (optional)

5. UGT-Glo™ Assay Protocol

Preparing the Luciferin Detection Reagent

1. Equilibrate the Luciferin Detection Reagent and the Reconstitution Buffer to room temperature.
2. Add entire contents of the bottle of Reconstitution Buffer to the amber bottle containing the Luciferin Detection Reagent. Mix by swirling or inverting the bottle several times. To avoid foaming of the reagent, do not vortex.
3. Remove the amount of reagent needed for the experiment to a separate tube. Add D-Cysteine, 100X, to a 1X final concentration to the same tube. Store at room temperature until ready for use

   Note: The reconstituted Luciferin Detection Reagent without D-Cysteine can be stored at room temperature for 24 hours or 4°C for 1 week without loss of activity. Store reagent without D-Cysteine at –20°C for up to 3 months. Be sure to mix the thawed Luciferin Detection Reagent well before use.

Preparing Assay Components

1. Thaw UGT and Control Membranes rapidly at 37°C. Once thawed, place immediately on ice. Mix well before using. Dispense unused membranes into single-use aliquots and store at –70°C. Avoid multiple freeze-thaw cycles.
2. Thaw UGT-Glo™ Buffer, UDPGA and UGT Multienzyme Substrate and keep at room temperature until use. Store any unused buffer, substrate and UDPGA at –20°C.

   Optional: If desired, alamethicin, a mixture of peptides for membrane permeabilization, may be added to the UGT-Glo™ Buffer, 5X, at a final concentration of 125µg/ml. We recommend adding the alamethicin from a 20mg/ml stock in methanol. The final concentration of alamethicin in the reaction will be 25µg/ml.
3. Prepare 20µl of 2X UGT reaction mixture for each well as indicated in Table 3. Determine the number of reactions to be set up, and add 1 or 2 reactions to compensate for pipetting errors. Dilute the 50mM UGT Multienzyme Substrate to 0.4mM in water immediately before adding it to the reaction mixture. Use Table 3 to calculate the amount of each component needed to prepare the 2X UGT reaction mixture. To avoid errors due to pipetting small volumes, prepare enough of the mixture for at least 10 wells.
4. Add components in order shown in Table 3 and store at room temperature until ready to use.

5. UGT-Glo™ Assay Protocol (continued)

Table 3. Preparation of the 2X UGT Reaction Mixture.

	Volume per Reaction	×	Number of Reactions	=	Total Volume
UGT-Glo™ Buffer, 5X	8.0µl				
UGT Multienzyme Substrate, diluted to 0.4mM	see Table 1				
UGT or Control Microsomes	see Table 1				
Water	up to 20µl				
Final Volume	20µl				

5. Prepare a separate 2X control reaction mixture for the minus-UGT control reactions by substituting Control Membranes for the UGT microsomes. To avoid errors due to pipetting small volumes, prepare enough of the mixture for at least 10 wells.
6. Prepare enough 16mM UDPGA (in water) to add 10µl to each well noted as plus-UDPGA.
7. Prepare test compounds at a 4X concentration. If test compounds are prepared from concentrated stock solutions that used a solvent other than water, an equivalent amount of vehicle must be included in the control reactions (minus-UGT control, untreated controls) to account for possible vehicle effects. Organic solvent concentrations should be kept to a minimum to avoid potential effects on UGT activity. UGT1A1 and UGT2B7 reactions with up to 4% DMSO added have consumption values that are ~80% of the same reactions without added solvent.

Performing the UGT-Glo™ Assay

If using 384-well plates instead of 96-well plates, we recommend using half the volumes indicated in the following protocol steps. However, the reactions may take longer in than in 96-well plates. To minimize this incubation difference, equilibrate the plates and the reagents to 37°C before assembling the reaction.

1. Add 10µl of 4X test compounds to the appropriate wells of the 96-well plate. Add water or vehicle to the control wells.
2. Add 10µl of 16mM UDPGA to shaded regions of the 96-well plate (Figure 7). Add 10µl of water to the unshaded wells.
3. Add 20µl of the 2X control reaction mixture to the control wells. Add 20µl of the 2X UGT reaction mixture to the rest of the wells.
4. Mix briefly on a plate shaker or by tapping the plate.

5. Incubate at 37°C for the desired amount of time (see Table 1).

6. Add 40µl of reconstituted Luciferin Detection Reagent plus D-Cysteine to all wells. Mix briefly on a plate shaker or by tapping the plate.

7. Incubate at room temperature for 20 minutes to stabilize the luminescent signal.

8. Record luminescence using a plate-reading luminometer or CCD camera. Values are displayed as relative light units (RLU).

   Note: Luminometer settings will depend on the manufacturer. Use an integration time of 0.25–1 second per well as a guideline. Relative light units (RLU) are arbitrary units that vary between instrument manufacturers and models. Do not expect readings from one luminometer to match those from a different instrument model.

	1	2	3	4	5	6	7	8	9	10	11	12
A	Minus-UGT Control	Minus-UGT Control	Minus-UGT Control	TC 7	TC 7	TC 7	Minus-UGT Control	Minus-UGT Control	Minus-UGT Control	TC 7	TC 7	TC 7
B	Untreated	Untreated	Untreated	TC 8	TC 8	TC 8	Untreated	Untreated	Untreated	TC 8	TC 8	TC 8
C	TC 1	TC 1	TC 1	TC 9	TC 9	TC 9	TC 1	TC 1	TC 1	TC 9	TC 9	TC 9
D	TC 2	TC 2	TC 2	TC 10	TC 10	TC 10	TC 2	TC 2	TC 2	TC 10	TC 10	TC 10
E	TC 3	TC 3	TC 3	TC 11	TC 11	TC 11	TC 3	TC 3	TC 3	TC 11	TC 11	TC 11
F	TC 4	TC 4	TC 4	TC 12	TC 12	TC 12	TC 4	TC 4	TC 4	TC 12	TC 12	TC 12
G	TC 5	TC 5	TC 5	TC 13	TC 13	TC 13	TC 5	TC 5	TC 5	TC 13	TC 13	TC 13
H	TC 6	TC 6	TC 6	TC 14	TC 14	TC 14	TC 6	TC 6	TC 6	TC 14	TC 14	TC 14

TC = test compound

7989MA

Figure 7. Plate layout for the UGT-Glo™ Assays. In this plate layout, all shaded wells receive UDPGA, while unshaded wells do not. The minus-UGT control is the UGT reaction set up with Control Membranes. The untreated sample is the UGT reaction with only compound vehicle present. TC 1–TC 14 are various compounds to be tested for their ability to modulate UGT activity.

6. Results and Data Analysis

1. Shown below is example UGT-Glo™ Assay data of a drug titration using UGT2B7. For each condition, there are three data points for the minus-UDPGA samples and three data points for the plus-UDPGA samples.

	Minus-UDPGA (RLU)			Plus-UDPGA (RLU)		
Control	773,705	753,202	754,208	707,516	698,734	724,197
UGT2B7 (no drug)	750,328	737,278	769,059	506,162	504,956	516,738
0.5µM drug	742,906	729,844	755,174	506,214	512,924	504,081
4.6µM drug	746,900	733,760	765,349	528,171	534,602	524,685
41µM drug	748,972	738,398	734,421	571,019	577,136	578,137
370µM drug	732,134	725,316	730,773	630,764	636,928	610,570

2. The Control Membranes controls account for the difference in luminescence due to the presence of 4mM UDPGA. To correct for this effect, subtract the average of the control plus-UDPGA values from the average of the minus-UDPGA values (in this example, 50,223RLU). Add this amount to all of the remaining plus-UDPGA values.

3. Subtract each of the three plus-UDPGA data points from the corresponding average minus-UDPGA values to give three differences for each condition. The magnitude of this difference is proportional to the UGT activity in the reaction. An example with the UGT2B7 no-drug data is shown below.

   Average UGT2B7 minus-UDPGA value = 752,221

   Difference_1 = 752,221 – 556,385 = 195,836

   Difference_2 = 752,221 – 555,179 = 197,042

   Difference_3 = 752,221 – 566,961 = 185,260

	Plus-UDPGA Values Subtracted from the Average Minus-UDPGA Data Values		
UGT2B7 (no drug)	195,836	197,042	185,260
0.5µM drug	186,204	179,494	188,337
4.6µM drug	170,276	163,845	173,735
41µM drug	119,355	113,238	112,237
370µM drug	48,421	42,257	68,615

4. To convert the calculated difference into percent of substrate consumed, divide each ΔRLU value by the average minus-UDPGA value for each condition and multiply by 100.

	Percent Substrate Consumed			Average (%)	CV (%)
UGT2B7 (no drug)	26.0	26.2	24.6	25.6	0.9
0.5µM drug	25.1	24.2	25.4	24.9	0.6
4.6µM drug	22.7	21.9	23.2	22.6	0.7
41µM drug	16.1	15.3	15.2	15.5	0.5
370µM drug	6.6	5.8	9.4	7.3	1.9

$$\textbf{\% Substrate Consumed} = \frac{\text{Background Corrected Difference}}{\text{Average Minus-UDPGA Value}} \times 100$$

CV = Standard Deviation (Substrate$_1$, Substrate$_2$, Substrate$_3$)

Changes from the average percent consumed of the untreated UGT reactions represent modulation of UGT activity. In this case, the drug was an inhibitor of UGT2B7. The data points above plus five others in the 0–3mM concentration range were plotted and fitted to a sigmoidal dose response curve using GraphPad Prism® 4.0. The IC_{50} for this drug was estimated to be 63µM.

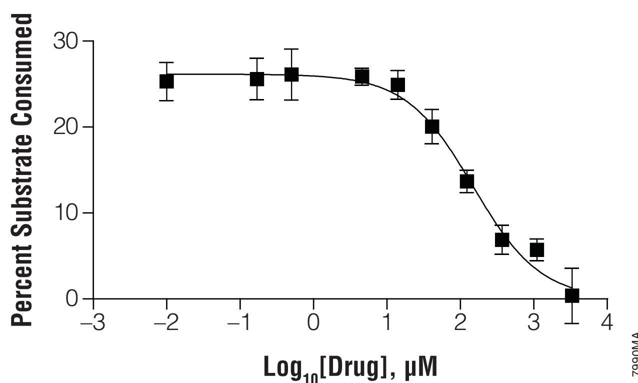

Figure 8. Example curve for the inhibition of UGT2B7.

7. Appendix

7.A. Composition of Buffers and Solutions

UGT Buffer, 5X

250mM TES (pH 7.5)
40mM magnesium chloride

UDPGA, 50mM

50mM UDPGA diluted in luciferin-free water

UGT Multienzyme Substrate

50mM UGT Multienzyme Substrate diluted in DMSO

UGT1A4 Substrate

50mM UGT1A4 Substrate diluted in DMSO

D-Cysteine, 100X

2M D-Cysteine hydrochloride monohydrate diluted in luciferin-free water

7.B. References

1. Zhang, D. *et al*. (2005) In vitro inhibition of UDP glucuronosyltransferases by atazanavir and other HIV protease inhibitors and the relationship of this property to in vivo bilirubin glucuronidation. *Drug Metab. Disp*. **33**, 1729–39.

2. Sakaguchi, K. *et al.* (2004) Glucuronidation of carboxylic acid containing compounds by UDP-glucuronosyltransferase isoforms. *Archives Biochem. Biophys*. **424**, 219–25.

3. Uchaipichat, V. *et al.* (2004) Human UDP-glucuronosyltransferases: Isoform selectivity and kinetics of 4-methylumbelliferone and 1-naphthol glucuronidation, effects of organic solvents, and inhibition by diclofenac and probenicid. *Drug Metab. Dispos.* **32**, 413–23.

7.C. Measuring the Affinity of Known Inhibitors

The ability of the UGT-Glo™ Assay to detect the effect of test compounds on UGT activity was demonstrated by performing the assay in the presence of known substrates and/or inhibitors (Figure 9). Lopinavir is an HIV protease inhibitor shown to inhibit UGT1A1 and UGT1A4, while having little effect on UGT2B7. Diclofenac is a nonsteroidal anti-inflammatory drug that is metabolized by most of the UGT isozymes. In Table 4, the K_m or K_i values obtained with the UGT-Glo™ Assay are compared to data from the literature. K_i values were calculated from both literature IC_{50} and UGT-Glo™ IC_{50} values using the equation $K_i = IC_{50}/(1+[S]/K_m)$.

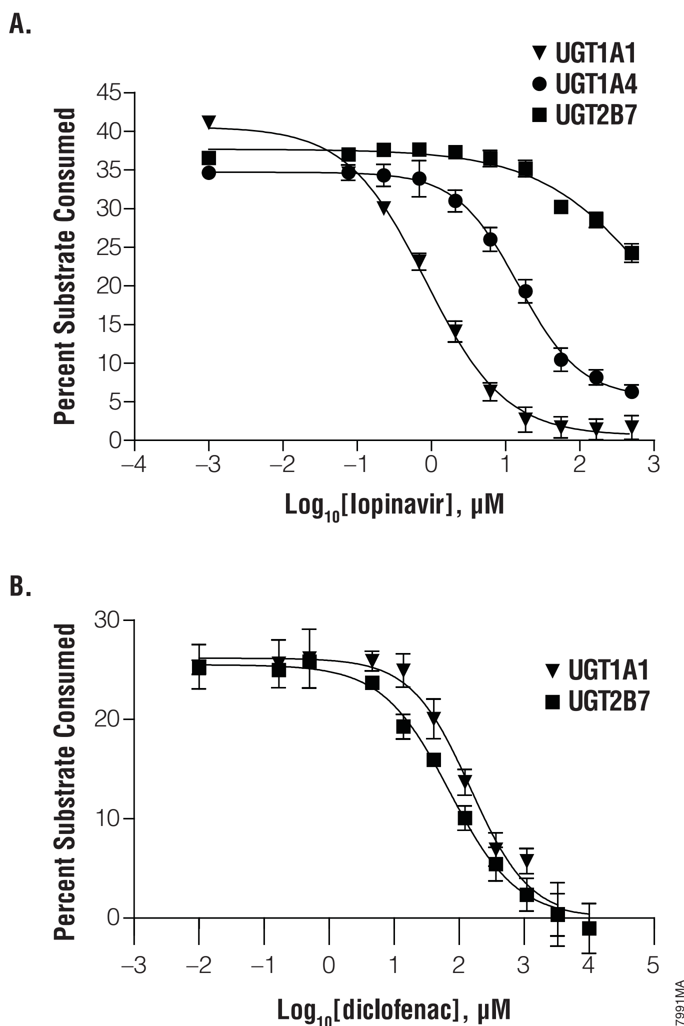

Figure 9. The UGT-Glo™ Assay can measure the activity of known UGT substrates and inhibitors with various isoforms. Panel A. The activity of UGT1A1, UGT1A4 and UGT2B7 were measured over a concentration range of 0–500µM of lopinavir. Final ethanol concentration was 0.5% in all reactions. UGT1A1 activity was measured using 20µM of the Multienzyme Substrate and 0.2mg/ml of enzyme for 45 minutes at 37°C. UGT1A4 activity was measured using 50µM of UGT1A4 Substrate and 0.4 mg/ml of enzyme for 90 minutes at 37°C. UGT2B7 activity was measured using 20µM of the Multienzyme Substrate and 0.1mg/ml of enzyme for 45 minutes at 37°C. RLU values were background-subtracted before conversion to consumption values. **Panel B.** The activity of UGT1A1 and UGT2B7 was measured over a concentration range of 0–3.3mM of diclofenac. Both isozymes were assayed using 20µM of multienzyme substrate and 0.1mg/ml of enzyme, and incubated at 37°C for 45 minutes. RLU values were background-subtracted before conversion to consumption values.

7.C. Measuring the Affinity of Known Inhibitors (continued)

Table 4. K_i or K_m Values for Known Substrates or Inhibitors.

Enzyme	Inhibitor	Reported IC_{50} (µM)	K_i or K_m Value (µM)	UGT-Glo™ Assay IC_{50} (µM)	UGT-Glo™ Assay Calculated K_i (µM)
UGT1A1	Lopinavir	8.61 (1)	4.3	0.84	0.42
UGT1A4	Lopinavir	7.61 (1)	3.8	14.8	8.6
UGT2B7	Lopinavir	>100 (1)	>50	>100	>50
UGT1A1	Diclofenac	na	52 ± 2 (3)	138	69
UGT2B7	Diclofenac	na	25 ± 12 (2)	62	31

na = not applicable

7.D. Using the UGT-Glo™ Assay to Analyze UGT Activity in Tissue Microsomes

The UGT-Glo™ Assay can be used to assess UGT activity and the effect of potential inhibitors in tissue microsome samples, such as human liver microsomes (HLM) and human intestinal microsomes (HIM). Because these tissue samples include multiple UGT isozymes at unknown expression levels, these enzyme sources tend to metabolize the Multienzyme Substrate at a much faster rate than single, recombinant UGTs (supersomes). The percent of Multienzyme Substrate consumed by HLM and HIM over time at various enzyme concentrations is shown in Figure 10. Use of the UGT1A4 substrate with tissue microsomes usually requires more enzyme and longer incubation times because it is measuring primarily UGT1A4 activity (data not shown). UGT activity was also detected in various rat and mouse microsomes, as well as human renal microsomes (data not shown).

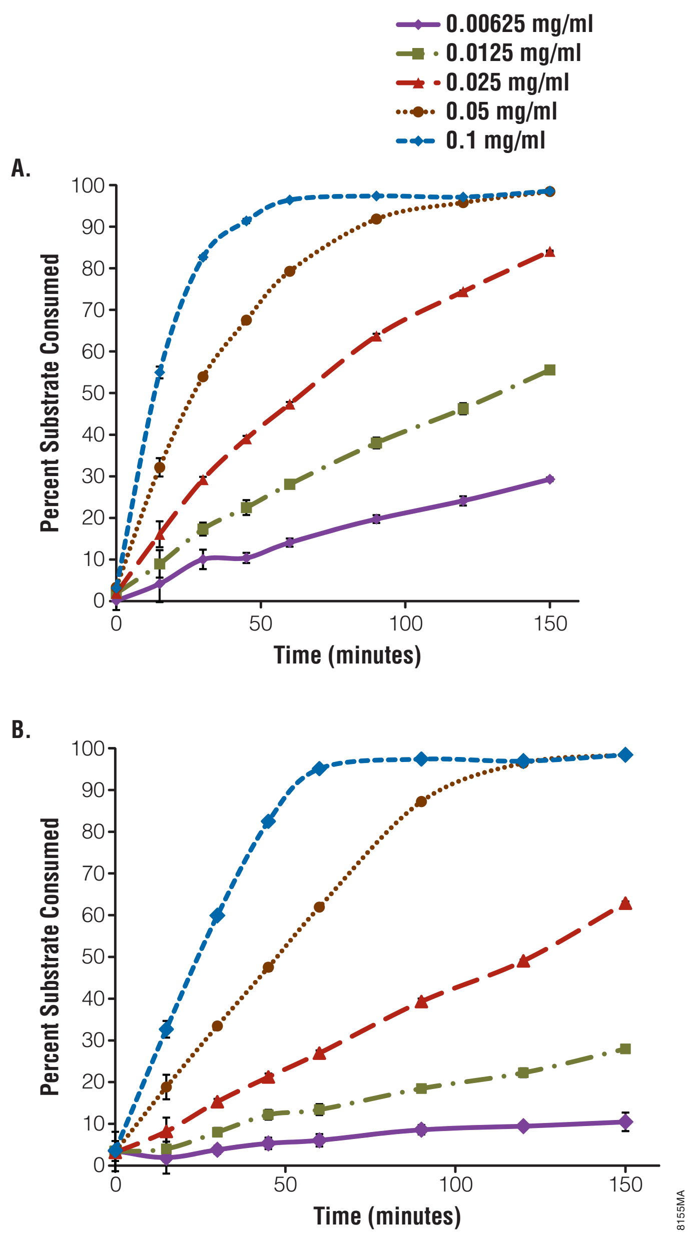

Figure 10. The UGT-Glo™ Assay can measure UGT enzyme activity in animal microsomes. Panel A. Forty microliter UGT-Glo™ reactions were performed using 50µM UGT Multienzyme Substrate and various concentrations of human liver microsomes (Xenotech, LLC Cat.# H0610). Enzyme concentration during the reaction is specified on the figure. **Panel B.** Same assay conditions as Panel A except human intestinal microsomes (Xenotech, LLC Cat.# H0610.I) were used as the source of enzyme.

8. Troubleshooting

For questions not addressed here, please contact your local Promega Branch Office or Distributor. Contact information available at: **www.promega.com**. E-mail: **techserv@promega.com**

Symptoms	Causes and Comments
High background luminescence	Luciferin contamination of one or more of the reaction components. Avoid workspaces and pipettes that are used with luciferin-containing solutions, including luminescence-based cell viability, apoptosis or gene reporter assays. Decontaminate work surfaces by wiping with a detergent solution or ethanol and rinsing with clean water. Rinse pipettes and other labware with distilled water at least three times. For automated dispensing systems, replace any components that have been used to dispense luciferin-containing solutions.
Little or no luminescent signal	D-Cysteine was not added to the Luciferin Detection Reagent before use. Be sure to add D-Cysteine to the Luciferin Detection Reagent before adding to the UGT reactions. If Luciferin Detection Reagent was added without D-Cysteine, add 4µl of 20X D-Cysteine to each well, mix and incubate for 20 minutes at room temperature before reading.
Unexpected inhibition of UGT enzymes	Inhibition of UGT by test compound vehicle. Compare the percent consumption of the minus-compound reactions with and without vehicle. A decrease in percent consumption in the presence of the vehicle indicates inhibition of UGT. Minimize solvent concentration or use a different solvent to dissolve test compounds.
Precipitation of reconstituted Luciferin Detection Reagent after storage at –20°C	Storage of the Luciferin Detection Reagent containing D-Cysteine can result in precipitation after freezing and thawing. Do not add D-Cysteine to the entire bottle of Luciferin Detection Reagent unless using the entire bottle for assays that day. Prepare only enough of the Luciferin Detection Reagent plus D-Cysteine for use within a few hours, and store excess Luciferin Detection Reagent **without D-Cysteine** at –20°C. Once D-Cysteine has been added to the Luciferin Detection Reagent, keep reagent at room temperature and use the same day. Discard any leftover reagent.

Symptoms	Causes and Comments
Minus-UDPGA RLU values decrease with increasing amounts of test compound	The test compound or its solvent are interfering with luciferase detection of the luciferin derivative. This can be quite common for highly colored compounds at high concentration. Normalize the RLU differences to percent of starting value to compensate for this effect.
Unexpected inhibition of UGT-Glo™ Assay	Luciferase inhibition. A luciferase enzyme is used to generate luminescence in UGT-Glo™ Assays. The potential for inhibition of luciferase has been minimized by maintaining high enzyme concentrations and using chemistries that reduce the effects of potential inhibitors. To test for luciferase inhibition, assemble two reactions, one with equal volumes of reconstituted Luciferin Detection Reagent and 400nM beetle luciferin (Cat.# E1601), and a second reaction with equal volumes of reconstituted Luciferin Detection Reagent and 400nM beetle luciferin plus test compound. Incubate the reactions for 10 minutes at room temperature, then measure luminescence. A decrease in luminescence in the presence of test compound indicates luciferase inhibition.
Lower-than-expected amounts of substrate consumed by UGT	Data in this publication are examples of single lots of UGT Supersomes™ (BD Gentest). Different lots of Supersomes™ or other sources of UGT enzyme may require more or less total membrane protein and more or less time to achieve the desired percent substrate consumed. If consumption is too low, increase time or amount of enzyme, making sure to verify that you are still under linear reaction conditions (<50% substrate consumed for most UGT enzymes).
	UGT enzyme preparations losing activity. Avoid multiple freeze-thaw cycles; thaw enzymes rapidly and keep on ice until ready for use.
Test compound IC_{50} values higher than expected	Verify that consumption of the substrate by the enzyme is linear with respect to time under the reaction conditions used (usually <50% substrate consumed for most enzymes). Running inhibition curves under nonlinear conditions results in overestimated compound IC_{50} values.

9. Related Products

Product	Size	Cat.#
P450-Glo™ CYP3A4 Assay (Luciferin-PPXE) DMSO-Tolerant Assay	10ml	V8911
	50ml	V8912
P450-Glo™ CYP3A4 Assay (Luciferin-PFBE) Cell-Based/Biochemical Assay	10ml	V8901
	50ml	V8902
P450-Glo™ CYP1A1 Assay	10ml	V8751
	50ml	V8752
P450-Glo™ CYP1B1 Assay	10ml	V8761
	50ml	V8762
P450-Glo™ CYP1A2 Assay	10ml	V8771
	50ml	V8772
P450-Glo™ CYP2C8 Assay	10ml	V8781
	50ml	V8782
P450-Glo™ CYP2C9 Assay	10ml	V8791
	50ml	V8792
P450-Glo™ CYP3A4 Assay	10ml	V8801
	50ml	V8802
P450-Glo™ CYP3A7 Assay	10ml	V8811
	50ml	V8812
P450-Glo™ CYP2C19 Assay	10ml	V8881
	50ml	V8882
P450-Glo™ CYP2D6 Assay	10ml	V8891
	50ml	V8892
P450-Glo™ CYP3A4 Screening System (Luciferin-PPXE) DMSO-Tolerant Assay	1,000 assays	V9910
P450-Glo™ CYP1A2 Screening System	1,000 assays	V9770
P450-Glo™ CYP2C9 Screening System	1,000 assays	V9790
P450-Glo™ CYP3A4 Screening System	1,000 assays	V9800
P450-Glo™ CYP2C19 Screening System	1,000 assays	V9880
P450-Glo™ CYP2D6 Screening System	1,000 assays	V9890
Luciferin-NAT2	3mg	P1721
Luciferin-3A7	3mg	P1741
Luciferin-4A	3mg	P1621
Luciferin-4F2/3	3mg	P1651
Luciferin-4F12	3mg	P1661
Luciferin-2J2/4F12 (ester)	3mg	P1671
Luciferin-MultiCYP (ester)	3mg	P1731

Promega

Product	Size	Cat.#
MAO-Glo™ Assay	200 assays	V1401
	1,000 assays	V1402
MAO-Glo™ Assay with MAO-A	1,000 assays	V1560
MAO-A	500µl	V1452
Pgp-Glo™ Assay System	10ml	V3591
Pgp-Glo™ Assay System with P-glycoprotein	10ml	V3601

Luminometers

Product	Size	Cat.#
GloMax®-Multi Detection System	1 each	E7031
GloMax® 20/20 Luminometer	1 each	E5311
GloMax® 96 Microplate Luminometer	1 each	E6501

[a]U.S. Pat. Nos. 6,602,677 and 7,241,584, European Pat. No. 1131441, Japanese Pat. Nos. 4537573 and 4520084 and other patents pending.

[b]The method of recombinant expression of *Coleoptera* luciferase is covered by U.S. Pat. Nos. 5,583,024, 5,674,713 and 5,700,673.

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