Version 3c Last updated 12 March 2026

ab273331
UGT Activity Assay / Ligand Screening Kit (Fluorometric)

View Kit datasheet: https://www.abcam.com/ab273331
(use https://www.abcam.cn/ab273331 for china, or
https://www.abcam.co.jp/ab273331 for Japan)

For the determination of UGT activity in cell and tissue fractions and screening for UGT drugs and ligands.

This product is for research use only and is not intended for diagnostic use.

Table of Contents

1. Overview

UGT Activity Assay / Ligand Screening Kit (Fluorometric) (ab273331) enables rapid measurement of native or recombinant UGT activity in biological samples such as liver microsomes and can also be used to assess the effect of drugs and other novel compounds on UGT activity. The assay utilizes a highly fluorescent UGT substrate with a large Stokes shift (Ex/Em = 415/502 nm) that allows determination of UGT activity by tracking the drop in fluorescence emission as the substrate is converted into a non-fluorescent glucuronide. The multi-isozyme substrate is glucuronidated by virtually all of the pharmacologically-relevant mammalian UGT1A and UGT2B enzymes. UGT specific activity is calculated by comparing the fluorescence loss versus a control reaction performed in the absence of the required cofactor UDPGA. The kit includes the pore-forming peptide antibiotic Alamethicin, which allows the UGT Substrate and UDPGA to rapidly diffuse across lipid membranes to access the UGT active site located in the lumen of microsomes. For verification of modulation of UGT activity by test ligands, diclofenac, a competitive inhibitor of most human and rodent UGT isozymes, is also included. The assay is highly sensitive, simple to perform and high-throughput adaptable. This assay can detect less than 0.1 mU UGT activity in biological samples. The kit contains a complete set of reagents sufficient for performing 100 reactions at a 100 µl reaction volume.

2. Protocol Summary

Prepare microsomal fractions

Prepare all reagents and standards as directed

Prepare the various Reaction Mixes as directed

Add Reaction Mixes to appropriate wells and incubate for 5 minutes at 37°C protected from light

Start reaction by addition of 5X UDPGA Substrate to wells

Immediately measure fluorescence (Ex/Em = 415/502 nm) in kinetic mode for 30-40 minutes at 37°C.

3. Precautions

Please read these instructions carefully prior to beginning the assay.

- All kit components have been formulated and quality control tested to function successfully as a kit.
- We understand that, occasionally, experimental protocols might need to be modified to meet unique experimental circumstances. However, we cannot guarantee the performance of the product outside the conditions detailed in this protocol booklet.
- Observe good laboratory practices. Gloves, lab coat, and protective eyewear should always be worn. Never pipette by mouth. Do not eat, drink or smoke in the laboratory areas.
- If applicable, please refer to the current Safety Data Sheet (SDS) provided with this product for safety, handling, and disposal information. The most up to date and current versions are available on our website https://www.abcam.com/en-us.

4. Storage and Stability

Store kit at -20°C in the dark immediately upon receipt.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in the Materials Supplied section.

Aliquot components in working volumes before storing at the recommended temperature.

5. Limitations

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

6. Materials Supplied

Item	Quantity	Storage temperature (before prep)
UGT Assay Buffer	100 mL	-20°C
UGT Inhibitor	1 vial	-20°C
UDPGA Stock	1 vial	-20°C
Alamethicin	50 µL	-20°C
UGT Substrate	1 vial	-20°C
UGT Positive Control	1 vial	-20°C

PLEASE NOTE: UGT Inhibitor was previously labelled as UGT Inhibitor (Diclofenac)(Lyophilized). The composition has not changed.

7. Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully perform this assay:

- Multiwell fluorescence microplate top reader
- Precision multi-channel pipette and reagent reservoir
- Anhydrous (reagent grade) DMSO
- dH_2O
- Black 96-well plates with flat bottom

8. Technical Hints

- **This kit is sold based on number of tests. A "test" simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.**
- Selected components in this kit are supplied in surplus amount to account for additional dilutions, evaporation, or instrumentation settings where higher volumes are required. They should be disposed of in accordance with established safety procedures.
- Avoid foaming or bubbles when mixing or reconstituting components.
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions.
- Ensure plates are properly sealed or covered during incubation steps.
- Ensure all reagents and solutions are at the appropriate temperature before starting the assay.
- Samples generating values that are greater than the most concentrated standard should be further diluted in the appropriate sample dilution buffer.
- Make sure all necessary equipment is switched on and set at the appropriate temperature.

9. Reagent Preparation

Briefly centrifuge small vials at low speed prior to opening.

9.1 **UGT Inhibitor:**

Reconstitute in 550 µL of dH_2O and vortex until fully dissolved to yield a 5 mM solution of diclofenac sodium. The solution should be stored at -20°C until use and is stable for at least 3 freeze/thaw cycles**.**

9.2 **UDPGA Stock (50X):**

Reconstitute with 220 µL dH_2O to yield a 50X stock solution. Aliquot the stock solution as desired and store the aliquots at -20°C. Avoid repeated freeze/thaw cycles and keep on ice while in use.

9.3 **Alamethicin:**

Alamethicin is provided as a solution in DMSO. Warm the solution to RT to melt the DMSO and vortex to ensure Alamethicin is completely dissolved. Aliquot and store at -20°C, stable for at least 3 freeze/thaw cycles.

9.4 **UGT Substrate:**

Reconstitute with 110 µL reagent-grade DMSO and vortex until fully dissolved to obtain a 250X stock solution. The UGT Substrate should be stored at -20°C and is stable for at least 3 freeze/thaw cycles. Allow the vial to warm to RT before opening and promptly retighten cap after use to avoid absorption of airborne moisture.

9.5 **UGT Positive Control:**

Do not reconstitute until ready to use. Reconstitute with 22 µL UGT Assay Buffer and mix thoroughly to ensure a homogenous solution (the concentrated solution will be slightly viscous and have an opaque, milky appearance). The reconstituted UGT Positive Control may be aliquoted and stored at -80°C. Avoid repeated freeze/thaw cycles and use aliquots within one month. Thaw aliquots rapidly at 37°C and place on ice until use (thawed aliquots should be used within 4 hours).

10. Sample Preparation

Standardized microsomal preparations may be purchased commercially (e.g. donor-pooled human liver microsomes) or prepared from tissue or cultured cells using commercial microsome isolation kits.

Alternatively, a crude enriched lysate can be prepared as detailed below.

Crude microsome-enriched lysate:

10.1 Start with ~50 mg tissue or ~5 x 10^6 pelleted, pre-washed cells and homogenize in 500 µL ice-cold UGT Assay Buffer with a Dounce homogenizer.

10.2 Incubate the homogenate on ice for 5 min and then centrifuge at 15,000 x g for 15 min at 4°C.

10.3 Collect the resultant clarified supernatant for the assay in a new pre-chilled microfuge tube and store on ice until use (cell and tissue lysates can also be stored at -80°C in aliquots for future experiments).

11. Standard Curve

11.1 Add 5 µL of the 250X UGT Substrate stock to 495 µL UGT Assay Buffer to generate a 0.1 nmole/µL UGT Substrate standard.

11.2 Add 0, 2, 4, 6, 8, 12, 16 and 20 µL of the 0.1 nmole/µL solution into a series of wells in a black 96-well plate, yielding 0, 0.2, 0.4, 0.6, 0.8, 1.2, 1.6 and 2.0 nmole/well fluorescence standard.

11.3 Adjust the volume of each well to 100 µL with UGT Assay Buffer.

Standard #	0.1 nmole/µL UGT Substrate Standard (µL)	UGT Assay Buffer (µL)	Fluorescence Standard (nmol/well)
1	20	80	2
2	16	84	1.6
3	12	88	1.2
4	8	92	0.8
5	4	96	0.4
6	2	98	0.2
7	0	100	0

12. Assay Procedure

Thaw all reagents thoroughly and mix gently.

2X Sample Premix:

12.1.1 Prepare a 2X Sample Premix by diluting the sample with UGT Assay Buffer.

12.1.2 For liver microsomes, we recommend that the 2X Sample Premix contain 0.05-0.2 mg protein/mL (resulting in a 0.025-0.1 mg/mL final concentration).

12.1.3 For each well, 50 µL of 2X Sample Premix should be prepared (however, we recommend preparing enough of the premix for at least 10 wells to prevent pipetting inaccuracies).

12.1.4 If using the UGT Positive Control, prepare a 2X Positive Control Premix solution by adding 10 µL of the reconstituted stock to 490 µL UGT Assay Buffer (a 1:50 dilution).

12.1.5 Place the 2X Sample Premix (and UGT Positive Control Premix, if applicable) on ice and add 5 µL of Alamethicin per ml to each of the 2X Sample Premix solutions (1 µL Alamethicin per 200 µL of premix solution) and incubate on ice for 15 min.

12.1.6 If desired, UGT activity in presence of test ligands may be measured. Test ligands should be dissolved into proper solvent to produce stock solutions. For each ligand, prepare a 5X solution by diluting in water or UGT Assay Buffer. We recommend running parallel solvent control well(s) to account for potential solvent effects. Final concentrations of organic solvents should be minimized to avoid impacting UGT activity; however DMSO has been shown to have little effect on UGT activity at a final concentration of ≤2% (v/v).

Δ **Note:** If assaying a crude tissue lysate or other uncharacterized sample, use the Bradford reagent or an equivalent protein assay to determine sample protein concentration prior to preparing the 2X Sample Premix.

Δ **Note:** Remember to account for any control reactions (such as the reaction blank, diclofenac inhibition and solvent control wells)

when calculating the amount of 2X UGT Sample Premix to prepare.

Δ Note: Dilute the UGT Positive Control directly before Alamethicin incubation. We do not recommend storing the diluted Positive Control.

12.1.7 Prepare a 10X working solution of UGT Substrate by diluting the 250X UGT Substrate stock in UGT Assay Buffer at a 1:25 ratio. Make a sufficient amount of 10X UGT Substrate solution to add 10 µL to each reaction to be performed (for 100 reactions, mix 40 µL of 250X UGT Substrate stock with 960 µL UGT Assay Buffer). The final concentration of UGT Substrate will be 40 µM, which in our experience is approximately equal to the Km in donor-pooled human liver microsomes.

12.1.8 Set up the assay reaction wells according to the table below. In addition to the test samples, prepare a reaction blank (no-UDPGA control) well. If desired, you may also prepare an inhibitor control well using the UGT Inhibitor solution (5 mM UGT Inhibitor, for a final concentration of 1 mM). Adjust the volume of test sample, inhibitor control and positive control wells to 80 µL/well with UGT Assay Buffer. For measurement of UGT activity in the presence of test ligands, replace Assay Buffer with 5X concentrated test ligand solution:

Component	Test Sample	+ Test Ligand	Blank (- UDPGA)	Positive Control
Sample Premix (2X)	50 µl	50 µl	50 µl	-
UGT Positive Control Premix (2X)	-	-	-	50 µl
UGT Substrate Working Solution (10X)	10 µl	10 µl	10 µl	10 µl
Diclofenac 5 mM Solution (5X)	-	-	-	-
UGT Assay Buffer	20 µl	-	40 µl	20 µl
Test Ligand (5X)	-	20 µl	-	-

12.1.9 Incubate the plate for 5 min at 37°C, protected from light. During the incubation, prepare a sufficient amount of 5X UDPGA solution to add 20 µl to each reaction well by diluting the 50X UDPGA Stock in UGT Assay Buffer at a 1:10 ratio.

12.1.10 Start the reaction by adding 20 µL of the 5X UDPGA solution to each well (except the no-UDPGA blank) using a multichannel pipette, yielding a final reaction volume of 100 µL per well.

12.1.11 Immediately measure the fluorescence at Ex/Em = 415/502 nm in kinetic mode for 30-40 min at 37°C. While the assay can be performed in either endpoint or kinetic mode, we strongly recommend reading in kinetic mode in order to ensure that the measurements recorded are within the linear range of the reaction. Ideal measurement time for the linear range may vary depending upon the amount and variety of active UGT isozymes in the sample.

Δ Note: Since the reaction starts immediately after the addition of the UDPGA, it is essential to preconfigure the fluorescence

microplate reader settings and use a multichannel pipette with a reagent reservoir to minimize lag time among wells.

Δ Note: The fluorescent UGT Substrate may exhibit photobleaching if exposed to overly rapid excitation light pulses. To minimize the likelihood of photobleaching, we recommend setting the microplate reader scan interval to 20 seconds or greater.

13. Calculations

13.1 For each reaction well, choose two time points (T_1 and T_2) in the linear phase of the reaction progress curves, obtain the corresponding fluorescence values at those points (RFU_1 and RFU_2) and determine the absolute value of the change in fluorescence over the time interval: $\Delta F = RFU_2 - RFU_1$

13.2 Calculate the specific fluorescence lost due to substrate glucuronidation (denoted by G) by subtracting the ΔF value of the blank (no UDPGA) reaction well from those of the test samples (S):

$$Gs = \Delta Fs - \Delta Fblank$$

Δ Note: The UGT Substrate is glucuronidated by the majority of characterized human hepatic and non-hepatic UGT isozymes, aside from UGT1A4. Thus, the calculated UGT activity represents a composite of all of the isozymes expressed in a particular sample. In human liver microsomes, the major isozymes include UGT1A1, 1A3, 1A6, 1A9 and 2B7.

UGT activity is obtained by applying the GS values to the substrate standard curve to get B nmole of substrate glucuronidated by sample UGT enzymes during the reaction time.

$$UDP\ Glucuronosyltransferase\ activity = \frac{B}{(\Delta t \times P)} \quad (nmol/min/mg)$$

B = Amount of substrate consumed, calculated from the Standard Curve (nmol)
Δt = Linear phase reaction time ($t_2 - t_1$) in minutes
P = Amount of protein in the well (in mg)

Unit definition:
One unit of UGT activity is the amount of enzyme that glucuronidates 1 µmole of fluorescent substrate per min (yielding a non-fluorescent conjugate) at 37°C and pH 7.5.

14. Typical Data

Typical standard curve – data provided **for demonstration purposes only**. A new standard curve must be generated for each assay performed.

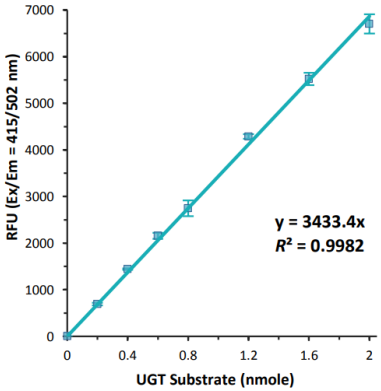

Figure 1. UGT Substrate Standard Curve. Glucuronidation of one mole of UGT Substrate produces a decline in fluorescence equivalent to the fluorescence generated by one mole of the unconjugated substrate.

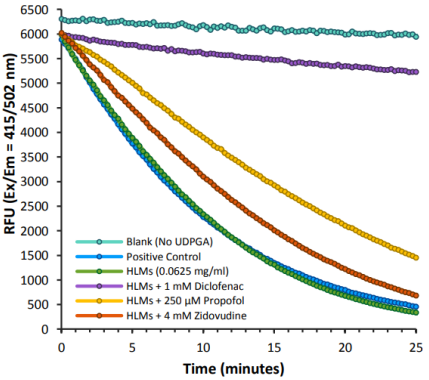

Figure 2. Reaction kinetics of fluorescent substrate glucuronidation in donor-pooled human liver microsomes (HLMs, 0.0625 mg/mL) at 37°C and inhibition of UGT activity in HLMs by the isozyme-selective ligands propofol (UGT1A9-selective) and zidovudine (UGT2B7-selective), as well as the non-selective UGT ligand diclofenac. For the blank reaction condition, vehicle (Assay Buffer) was substituted for the cofactor UDPGA.

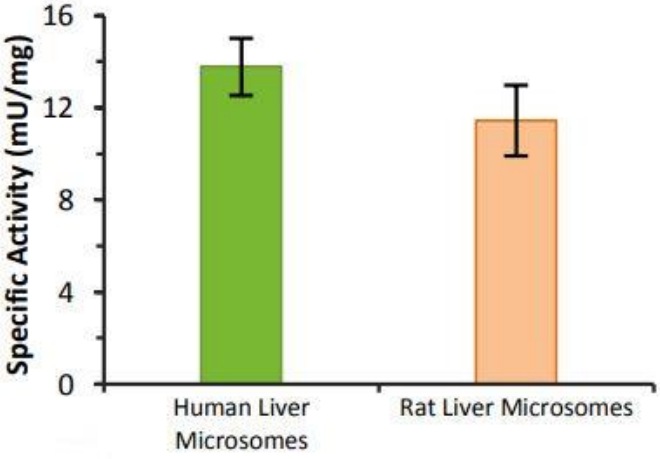

Figure 3. Specific UGT activity in pooled human and rat liver microsomes (mean ± SEM of 3 replicates).

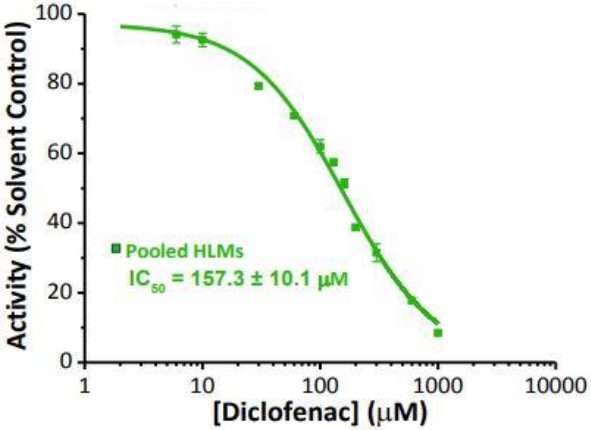

Figure 4. Dose-response curve for UGT inhibition by diclofenac in HLMs. Percent activity was calculated for each concentration by comparison to activity of reaction containing vehicle only. IC50 values were derived by 4-parameter logistic curve fitting with each point representing the mean ± SEM of at least 3 replicates. Assays were performed according to the kit protocol.

15. FAQ / Troubleshooting

General troubleshooting points are found at https://www.abcam.com/en-us/products/biochemical-assays.

16. Notes

Technical Support

For all technical or commercial enquiries please go to:

https://www.abcam.com/en-us/contact-us

https://www.abcam.cn/contact-us (China)

https://www.abcam.co.jp/contact-us (Japan)