UDP-glycosyltransferase BACULOSOMES® Transferase Activity Assay

UGT1A1, UGT1A3, UGT1A6, UGT1A7, UGT1A10, UGT2B7
Product # P2327, P2470, P2358, P2447, P2472, P2636

1.0 PURPOSE/DISCUSSION

The glucuronidation reactions catalyzed by human UDP-glycosyltransferase (UGT) enzymes play an important role in the metabolism of many drugs and other xenobiotics. UGT activity is defined by the transfer of glucoronic acid from UDP-glucuronic acid (UDPGA) to an aglycone substrate containing suitably reactive hydroxyl, sulfhydryl, aromatic amino and carboxylic acid moieties. UGTs are a group of integral membrane enzymes of the endoplasmic reticulum that have been divided into two families based on evolutionary divergence of their genes. UGTs of the 1A family play an important role in both the metabolism of dietary constituents, phenols, and therapeutic drugs, and also the glucuronidation of bilirubin and iodothyronines. UGTs of the 2B family are involved in the metabolism of bile acids, phenol derivatives, catechol-estrogens and steroids. Human UGT2B7 is one of the most important UGT isoforms. It glucuronidates opioids, hyodeoxycholic acid, catechol-estrogens, androsterone, and many non-steroidal anti-inflammatory drugs (NSAIDs) with high efficiency. Although it is widely recognized that the liver is the major site of glucuronidation, it is now clear that UGTs are also found in extra-hepatic tissues. To date, at least 20 human UGTs have been cloned and sequenced. Several of them have been expressed in heterologous systems and have had their substrate specificity determined.

2.0 SAFETY PRECAUTIONS

Normal precautions exercised in handling laboratory reagents should be followed. Refer to the Material Safety Data Sheet(s) (MSDS) for any updated risk, hazard, or safety information. The reagents supplied are not considered hazardous according to 29 CFR 1910.1200. The chemical, physical, and toxicological properties of these products may not, as yet, have been thoroughly investigated. We recommend using gloves, lab coats, and eye protection when working with any chemical reagents.

3.0 DESCRIPTION

3.1 Reagents Supplied

UDP-glycosyltransferase BACULOSOMES® Reagents are microsomes prepared from *Sf-9* insect cells that were infected with a recombinant baculovirus containing the cDNA for a specific human UGT. The substrates octyl gallate and α-naphthol can serve as general aglycone acceptors (probe substrates) for a variety of 1A family UGT enzymes using either TLC[1] or organic extraction assays[2]. Hyodeoxycholic acid may be used as a probe substrate for UGT2B7 organic extraction assays[2]. In addition to the probe substrates, PanVera uses a variety of aglycones in order to demonstrate the substrate specificity for each recombinant UGT isoform. Control BACULOSOMES® Reagents are prepared from the *Sf-9* insect cells infected with wild-type (non-recombinant) baculovirus. PanVera supplies a comprehensive panel of UGT products from both the 1A and 2B families including:

1A family

P2327	UGT1A1	BACULOSOMES® Reagent 5 mg
P2470	UGT1A3	BACULOSOMES® Reagent 5 mg
P2358	UGT1A6	BACULOSOMES® Reagent 5 mg
P2447	UGT1A7	BACULOSOMES® Reagent 5 mg
P2472	UGT1A10	BACULOSOMES® Reagent 5 mg

2B family

P2636	UGT2B7	BACULOSOMES® Reagent 5 mg

Control

P2448	*Sf*-9 Control	BACULOSOMES® Reagent 5 mg

3.2 Reagents Required but Not Supplied

- 1 M Tris-HCl (pH 7.5)
- 100 mM $MgCl_2$
- 100 mM *d*-saccharic acid 1,4-lactone
- Aglycone substrate stock solutions dissolved in methanol (10 mM octyl gallate, 100 mM α-naphthol)
- 1 mg/mL phosphatidyl choline dissolved in water
- 1 mM uridine 5′ diphosphoglucuronic acid (UDPGA)
- [^{14}C]UDPGA, 320 Ci/mol (DuPont, NEC414)
- 95% ethanol
- 0.7 M glycine-HCl (pH 2.0) containing 1% (v/v) Triton®X-100
- Ready Safe™ Liquid scintillation cocktail (Beckman)

4.0 EXPERIMENTAL DESIGN

The following is a suggested protocol for the measurement of UDP-glycosyltransferase activity. Two different methods are suggested for analyzing the transferase assay; both involve isolation of the [^{14}C]-labeled glucoronide reaction product. One method involves organic extraction and the other uses TLC to separate the glucuronidated metabolite from [^{14}C]UDPGA and the other reaction components.[1,2] The extraction method is much quicker to perform; however, when dealing with novel substrates, artifacts may be created by inefficient extraction of the glucuronidated substrate. An advantage to the TLC method is that the presence or absence of glucuronidated metabolites and [^{14}C]UDPGA can be visualized due to their different R_f values.

4.1 Procedure

In general, the optimal rate of glucuronidation will vary depending on the recombinant UGT that is being tested. It is recommended that the investigator test the linearity of the assay with different protein and substrate concentrations. Information on UGT assay linearity with various probe substrates is available from PanVera. A guide to the conditions routinely used is shown below:

Part #	BACULOSOME Enzyme	Substrate	Substrate Concentration	Protein Concentration	Time
P2327	UGT1A1	octyl gallate	100 µM final	0.4 mg/mL	10 min.
P2470	UGT1A3	octyl gallate	200 µM final	0.4 mg/mL	15 min.
P2358	UGT1A6	α-naphthol	100 µM final	0.4 mg/mL	20 min.
P2447	UGT1A7	octyl gallate	100 µM final	0.2 mg/mL	20 min.
P2472	UGT1A10	α-naphthol	1000 µM final	0.8 mg/mL	15 min.
P2636	UGT2B7	hyodeoxycholic acid	50 µM final	0.4 mg/mL	15 min.

We recommend using two negative controls for the assay:

1. An incubation in the absence of aglycone acceptor (substrate)
2. A control for endogenous insect cell glucuronidation activity using BACULOSOMES® Reagent prepared from *Sf-9* insect cells infected with a wild-type virus (Part # P2448).

Incubations:

For each 100 µL reaction make up two mixes:

Buffer Mix	
1 M Tris HCl, (pH 7.5)	10.0 µL
100 mM $MgCl_2$	10.0 µL
100 mM saccharolactone	10.0 µL

Substrate Mix	
cold UDPGA	8.0 µL
ddH_2O	7.8 µL
aglycone substrate	1.0 µL
phosphatidylcholine	10.0 µL
[^{14}C]UDPGA	3.2 µL

In a microcentrifuge tube, combine 30 µL of Buffer Mix and 40 µL of BACULOSOMES® Reagent. The concentration of BACULOSOMES® Reagent used in each assay will vary depending on the UGT enzyme used. Pre-incubate for 2-3 minutes at 37°C and then start the reaction by adding 30 µL of Substrate Mix. Incubate at 37°C for the appropriate time and terminate the reaction by adding either 100 µL of 0.7 M glycine-HCl (pH 2.0) containing 1% (v/v) Triton® X-100 (for extraction analysis), or 100 µL of pre-chilled 95% ethanol (for TLC analysis).

4.2 Analysis

TLC Analysis: Vortex the quenched reaction and precipitate the protein by centrifugation in a microcentrifuge for 10 minutes at maximum speed. Transfer the supernatant to a fresh tube. Perform TLC analysis using 40 µL of supernatant as described previously.[2] Quantitate by scintillation counting of radioactive spots after autoradiography. Glucuronides can be identified by incubation of reaction products without saccharolactone, a glucuronidase inhibitor, and inclusion of β-glucuronidase.

For organic extraction: Add 1 mL of water-saturated ethyl acetate and vortex the mixture for 5 minutes. Centrifuge the samples for 5 minutes in a microcentrifuge at maximum speed. Precipitated protein will appear at the interphase. Transfer a 200- µL aliquot of the upper ethyl acetate phase to a scintillation vial, add scintillation cocktail, and count radioactivity.[1]

5.0 REFERENCES

1. Matern, H., *et al.* (1994) *Anal. Biochem.* **219:** 182-188.
2. Bansal, S.K., *et al.* (1980) *Anal. Biochem.* **109:** 321-329.

6.0 BIBLIOGRAPHY

1. Burchell, B., *et al.* (1995) *Life Sciences* **57:** 1819-1831.
2. Mackenzie, *et al.* (1997) *Pharmacogenetics* **7:** 255-269.
3. Tephly, T.R, *et al.* (1998) *Adv. Pharmacol.* **42:** 343-346.