RECOMBINANT HUMAN UDP-GLUCURONOSYLTRANSFERASES (UGTS) ENZYMES

Product No.	Description
CYP200	Human UGT1A6
CYP201	Human UGT1A1
CYP202	Human UGT1A3
CYP203	Human UGT1A8
CYP204	Human UGT1A10
CYP205	Human UGT1A9
CYP206	Human UGT1A7
CYP207	Human UGT1A4
CYP209	Human UGT2B7
CYP210	Human UGT2B17
CYP208	Control Sf9 microsomes

PRODUCT DESCRIPTION:

Recombinant human UGT enzymes are supplied as microsomes, isolated from Sf9 insect cells (derived from *Spodoptera frugiperda*), following infection with recombinant baculovirus. The microsomes are supplied in 25 mM Tris-acetate buffer (pH 7.6) containing 10% (v/v) glycerol and 1 mM EDTA.

STORAGE: ≤ -80°C

MATERIALS

400 mM Tris-HCl, pH 7.50 at 37°C
100 mM $MgCl_2$
Deionized water
Substrate solution
20 mM UDPGA (cofactor) in water
Alamethicin, 1 mg/ml in water/methanol, 50/50 (v/v) (optional)
1 M HCl, methanol or acetonitrile as stop reagent

EQUIPMENT

Water bath set to 37°C
Suitable polypropylene tubes
Centrifuge

INCUBATION PROCEDURE:

Incubations are usually conducted in 100 mM Tris buffer, pH 7.5 (at 37°C). Other buffers may be used, but some UGTs exhibit lower activity in phosphate buffer. The cofactor UDP-glucuronic acid (UDPGA) is required for activity (recommended final concentration 2 – 5 mM). Stock solutions of UDPGA should be stored frozen, in aliquots. Alamethicin (10 µg/ml final concentration) can be used to overcome the latency of UGT, which can otherwise result in reduced activity for some substrates. If alamethicin is being used, it should be pre-incubated with the microsomes in the pre-mix on ice for 15 – 20 min before starting the assay.

DRUG METABOLISM

1) Thaw the insect cell microsomes and the cofactor solution on ice, and keep on ice once thawed. Mix the microsomes by gently vortexing just before use.
2) Prepare incubations on ice, using the guide below. Reactions are initiated by the addition of UDPGA, so this should be omitted at the preparation stage. Multiple incubations should be prepared from a pre-mix (see below).

For a single 0.2 ml incubation:

400 mM Tris-HCl pH 7.5 (at 37°C)	50 µl
100 mM $MgCl_2$	10 µl
Water	(120 – x – y) µl
Substrate / test compound	x µl
UGT	y µl
[20 mM UDPGA	20 µl (for 2 mM final concentration)] – add later

Pre-mix for 20 x 0.2 ml incubations:

400 mM Tris-HCl pH 7.5 (at 37°C)	1000 µl
100 mM $MgCl_2$	200 µl
Water	(2400 – xx – yy) µl
Substrate / test compound	xx µl
UGT	yy µl

The volume of substrate will be determined by the required final concentration. Solvent concentration (e.g. methanol, DMSO) should be kept to a minimum with a maximum concentration in the assay of 1% (v/v). As an example, adding 4 µl of a 20 mM stock solution to the above pre-mix will give a final substrate concentration of 20 µM in the test (after initiating the reactions by adding the cofactor, UDPGA).

The concentration of UGT will be dependent on the requirements of the assay and the activity of the enzyme with the substrate being used. Typical protein concentrations can be found on the data sheet accompanying the specific UGT product being used. It should be

borne in mind, however, that these concentrations are specific to the substrate being used and are set to minimise substrate loss (less than 10% across the assay). If you are looking for substrate loss in the assay then the concentration of UGT should be increased accordingly.

3) Add 180 µl of pre-mix to each assay tube (1.5 ml polypropylene microtubes work well) and pre-incubate at 37°C for 5 min. The assay volume can be adjusted as required: we also use 1 ml final volume assays in 15 ml polypropylene conical tubes, in which case 900 µl of pre-mix would be used per tube.
4) Add 20 µl of 20 mM UDPGA (100 µl for a 1 ml assay volume) to each tube to initiate the reaction, and incubate at 37°C (typically 5 – 30 minutes, but this depends on the UGT and substrate being used).
5) Stop the reaction(s) by the addition of one of the following:
   0.1 volumes 1 M HCl (20 µl for a 200 µl incubation)
   0.5 volumes acetonitrile (100 µl for a 200 µl incubation)
   1 volume methanol (200 µl for a 200 µl incubation)
6) Place the samples on ice for at least 10 minutes and then centrifuge: approximately 13,000 rpm for 10 mins for microtubes or 4,000 rpm for 20 mins for 15 ml tubes)
7) Recover the supernatants for further analysis.

CAUTION:

This product is being sold for research and/or manufacturing purposes only. The biological samples supplied by BioIVT, or any material isolated from the samples, are for in-vitro research use only and are not to be used as a source of material for clinical therapies. Human material may be used in vivo in animals. The user assumes all responsibility for its usage and disposal, in accordance with all regulations.