UDP-Glucuronosyltransferas（UDPGT）Activity Assay Kit

Detection Equipment: Spectrophotometer

Catalog Number: BC5815

Size: 100T/48S

Components:Please carefully check the volume of the reagent and the volume in the bottle before use. If you have any questions, please contact Solarbio staff in time.

Reagent name	Size	Preservation condition
Extract	Liquid 100 mL×1	2-8℃ storage
Reagent I	Liquid 15 mL×1	2-8℃ storage
Reagent II	Liquid 1.2 mL×1	2-8℃ storage
Reagent III	Liquid 1.2 mL×1	2-8℃ storage
Reagent IV	Liquid 1.2 mL×1	2-8℃ storage
Reagent V	Powder ×1	-20℃ storage
Reagent VI	Liquid 12 mL×1	2-8℃ storage
Standard	Liquid 1 mL×1	2-8℃ storage

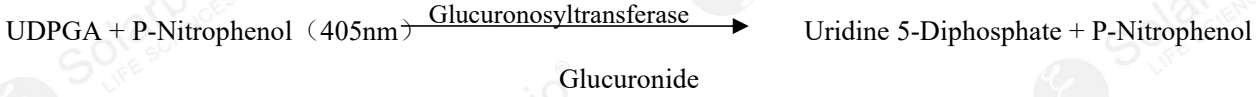
Solution reparation :

1. Reagent V: Add 0.619mL distilled water before use, pack at -20℃ can be stored for 4 weeks to avoid repeated freezing and thawing;
2. Working solution: prepared according to the proportion of reagent II: reagent III: reagent IV =100μL: 100μL: 100μL (0.3mL, 10T) according to the sample volume before use, and used now;
3. Standard: 5μmol/mL of p-nitrophenol solution. Before use, 50μL 5μmol/mL p-nitrophenol solution was added to 950μL distilled water to prepare 0.25μmol/mL p-nitrophenol solution, which was used for immediate preparation.

Description:

Glucuronosyltransferase (UDPGT/UGT, EC 2.4.1.17) is a membrane protein that binds to the endoplasmic reticulum and catalyzes the transfer of a glucuronate group from the donor uridine diphosphate glucuronate to the recipient. Receptors include phenols, alcohols, amines, and fatty acids. Glucuronidation metabolism promotes the excretion of drugs and other foreign substances through the kidney or bile, and plays an important role in the metabolism, detoxification and clearance of organisms.

UDPGT catalyzes the transfer of the glucuronic acid group of the donor uridine diphosphate glucuronic acid to p-nitrophenol, which has a characteristic absorption peak at 405nm. The UDPGT activity can be calculated by measuring the reduction rate of the absorbance value.



Note: Before the experiment, it is recommended to select 2-3 sample with large expected differences for pre-experiment. If the absorbance value of the sample is not within the measurement range, it is recommended to dilute or increase the sample size for detection.

Reagents and Equipment Required but Not Provided:

Spectrophotometer/microplate reader, cryogenic centrifuge, analytical balance, water bath / constant temperature incubator, micro glass cuvette/96 well flat-bottom plate, adjustable pipette, mortar/homogenizer, ice and distilled water.

Protocol:

I. Sample processing (The sample size to be tested can be appropriately adjusted, and the specific proportion can be referred to the literature)

1. Weigh 0.1g sample, add 1.0mL extract, homogenize on ice with homogenizer or mortar;
2. Centrifuge at 4℃ at 800g for 10 min, discard precipitation and retain supernatant; Centrifuge at 4℃ at 9000 g for 20 min, discard precipitation and retain supernatant;
3. Centrifuge at 12000g for 60 min at 4℃, discard supernatant and leave precipitation. 0.5mL of extraction solution was added to the precipitate and then placed on the ice to be measured.

II. Measurement Steps

1. It can be seen that the spectrophotometer is preheated for more than 30min, the wavelength is adjusted to 405nm, and zero it with distilled water.
2. Preheat Reagent I at 37℃ for 15min;
3. Operation table (Add the following reagents to the EP tube) :

Reagent name（μL）	Test tube	Control tube	Blank tube	Standard tube
Sample	50	50	-	-
Distilled water	-	-	50	-
Standard	-	-	-	50
Reagent I	110	120	150	150
Working solution	30	30	-	-
Reagent V	10	-	-	-
After mixing, reaction at 37℃ for 10min				
Reagent VI	100	100	100	100
Mix well, centrifuge at 4℃, 8000g for 10min, take the supernatant 200μL and apply it to the microglass colorimetric dish / 96-well plate, determine the light absorption value at 405nm, respectively recorded as A_{text}, $A_{control}$, A_{blank} and $A_{standard}$, calculate $\Delta A_{text}=A_{control}-A_{text}$, $\Delta A_{standard}=A_{standard}-A_{blank}$. Blank tube and standard tube only need to be tested 1-2 times.				

III. Calculations

1. Calculated by sample protein concentration

Definition of unit: The catalytic consumption of 1nmol p-nitrophenol per mg of hiprotein per minute is defined as one unit of enzyme activity.

$$\text{UDPGT activity (U/mg prot)} = (\Delta A_{text} \div \Delta A_{standard} \times C_{standard}) \times V_{sample} \div (Cpr \times V_{sample}) \times 10^3 \div T$$
$$= 25 \times \Delta A_{text} \div \Delta A_{standard} \div Cpr$$

2. Calculated by sample quality

Definition of unit: The catalytic consumption of 1nmol p-nitrophenol per g tissue per min is

defined as one unit of enzyme activity.

UDPGT activity (U/g mass) = ($\Delta A_{text} \div \Delta A_{standard} \times C_{standard}$)$\times V_{sample} \div (W \times V_{sample} \div V_{total\ sample}) \times 10^3 \div T$

$= 12.5 \times \Delta A_{text} \div \Delta A_{standard} \div W$

$C_{standard}$: 0.25 μmol/mL;

V_{sample}: The sample volume added to the reaction system, 0.05mL;

$V_{total\ sample}$: The volume of the extraction solution added by the suspension precipitation, 0.5mL;

Cpr: Protein concentration, mg/mL;

W: Sample quality, g;

10^3: Unit conversion factor, 1μmol=10^3nmol;

T: Reaction time, 10min.

Note:

1. If ΔA is less than 0.004 or the absorption value of the tube is close to the control, the sample size can be increased or the reaction time can be extended to 37℃ before the determination; If ΔA is measured greater than 0.9, it is recommended to dilute the suspension appropriately with the extract before measuring. Change the calculation formula simultaneously.

Experimental example:

1. Take 0.1027g fresh mouse liver sample, add 1mL extract solution for ice bath homogenization, add 0.5mL extract solution for re-suspension precipitation after multiple centrifugation, follow the measurement procedure, calculated by 96-well plate measurement: $\Delta A_{text} = A_{control} - A_{text}$ =0.970-0.524=0.446, $\Delta A_{standard} = A_{standard} - A_{blank}$ = 0.533- 0.036=0.497, calculated by sample quality:

   UDPGT activity (U/g mass) $= 12.5 \times \Delta A_{text} \div \Delta A_{standard} \div W$ =109.224 U/g mass

References：

[1] Visser TJ, Kaptein E, van Raaij JA. et al. Multiple UDP-glucuronyltransferases for the glucuronidation of thyroid hormone with preference for 3,3',5'-triiodothyronine (reverse T3) [J]. Federation of European Biochemical Societies Letters, 1993, 315(1): 65-68.

[2] Viollon-Abadie C, Lassere D, Debruyne E. et al. Phenobarbital, beta-naphthoflavone, clofibrate, and pregnenolone- 16alpha-carbonitrile do not affect hepatic thyroid hormone UDP-glucuronosyl transferase activity, and thyroid gland function in mice [J]. Toxicology and Applied Pharmacology, 1999, 155(1): 1-12.

[3] Nicod L, Rodriguez S, Letang JM. et al. Antioxidant status, lipid peroxidation, mixed function oxidase and UDP-glucuronyl transferase activities in livers from control and DOCA-salt hypertensive male Sprague Dawley rats [J]. Molecular and Cellular Biochemistry, 2000, 203(1-2): 33-39.

Related products:

BC5170/BC5175　直接胆红素（DBIL）含量检测试剂盒

BC5180/BC5185　总胆红素（TBIL）含量检测试剂盒