UDP-Glucuronosyltransferas（UDPGT）Activity Assay Kit

Detection Equipment: Spectrophotometer
Catalog Number: BC5810
Size: 50T/24S

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 50 mL×1	2-8℃ storage
Reagent I	Liquid 30 mL×1	2-8℃ storage
Reagent II	Liquid 2.4 mL×1	2-8℃ storage
Reagent III	Liquid 2.5 mL×1	2-8℃ storage
Reagent IV	Liquid 2.4 mL×1	2-8℃ storage
Reagent V	Powder ×1	-20℃ storage
Reagent VI	Liquid 25 mL×1	2-8℃ storage
Standard	Liquid 1 mL×1	2-8℃ storage

Solution reparation :

1. Reagent V: Add 1.238mL 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 =80μL: 80μL: 80μL (0.24mL, 2T) 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.

UDPGA + P-Nitrophenol（405nm） $\xrightarrow{\text{Glucuronosyltransferase}}$ Uridine 5-Diphosphate + P-Nitrophenol Glucuronide

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.

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Reagents and Equipment Required but Not Provided:

Spectrophotometer, cryogenic centrifuge, analytical balance, water bath/thermostatic incubator, 1mL glass cuvette, adjustable pipetting gun, 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	200	200	-	-
Distilled water	-	-	200	-
Standard	-	-	-	200
Reagent I	440	480	600	600
Working solution	120	120	-	-
Reagent V	40	-	-	-
After mixing, reaction at 37℃ for 10min				
Reagent VI	400	400	400	400
Mix well, centrifuge at 4℃, 8000g for 10min, take 1mL of supernatant in 1mL glass cuvea, 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) = ($\Delta A_{text} \div \Delta A_{standard} \times C_{standard}$)×$V_{sample} \div (W \times V_{sample} \div V_{total\ sample}) \times 10^3 \div T$
=12.5×$\Delta A_{text} \div \Delta A_{standard} \div W$

$C_{standard}$: 0.25 μmol/mL;

V_{sample}: The sample volume added to the reaction system, 0.2mL;

$V_{total\ sample}$: 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, and calculate with 1mL glass cuvette: $\Delta A_{text} = A_{control} - A_{text}$ =1.473-0.843=0.630, $\Delta A_{standard} = A_{standard} - A_{blank}$ = 0.741-0.005 =0.736, calculated by sample quality:
UDPGT activity (U/g mass) =12.5×$\Delta A_{text} \div \Delta A_{standard} \div W$ =104.184 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.

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