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β-Glucuronidase (β-GD) Activity Assay Kit

Note: Before the experiment, it is recommended to select 2-3 sample with large expected differences for pre-experiment.

Operation equipment: Spectrophotometer/Microplate reader

Cat No: AK0949-100T-48S

Size: 100T/48S

Product Composition: Before use, please carefully check whether the volume of the reagent is consistent with the volume in the bottle. If you have any questions, please contact salesman in time.

Reagent name	Size	Preservation Condition
Extract solution	Liquid 60 mL×1	2-8℃
Reagent I	Liquid 0.6 mL×1	2-8℃
Reagent II	Liquid 10 mL×1	2-8℃
Reagent III	Liquid 12 mL×1	2-8℃
Reagent IV	Liquid×1	2-8℃

Solution Preparation:

1. Standard solution : The standard is 5μmol/mL p-Nitrophenol. 20 μL 5 μmol/mL p-Nitrophenol was added to 980 μL distilled water to prepare 0.1 μmol/mL p-Nitrophenol standard solution.

Description:

β-glucuronidase (β-GD, EC 3.2.1.31). β-GD is a lysosomal acid hydrolase responsible for the catalytic depolymerization of β-D-glucuronide, which has become a feasible molecular target for a variety of therapeutic applications. The enzyme is widely distributed in mammalian tissues, body fluids, and microbiota, and can also be found in plants, fish, insects, and mollusks. For humans, the enzyme can be used as a reliable biomarker for tumor diagnosis and clinical treatment evaluation due to increased expression of β-GD in necrotic areas and other body fluids in patients with different forms of cancer, such as breast cancer, cervical cancer, colon cancer, lung cancer, kidney cancer and leukemia.

β-GD can catalyze the substrate 4-nitrophenyl-β-D-glucuronide to produce p-Nitrophenol, which has a characteristic absorption peak at 405 nm. Because the absorbance value of p-Nitrophenol is proportional to the content, the enzyme activity of β-glucuronidase can be calculated by measuring the content of p-Nitrophenol produced per unit time.

Reagents and Equipment Required but Not Provided:

Spectrophotometer/microplate reader, water-bath, desk centrifuge, adjustable pipette, micro glass cuvette/96 well plate, mortar/ homogenizer/cell ultrasonic crusher, ice and distilled water.

Operation procedure:

I. Sample preparation

1. Tissue sample: Add extract solution according to the ratio of tissue mass (g) : Extract solution (mL)= 1:5 ~10 (it is recommended to weigh 0.1g sample and add 1.0mL extract solution), after ice bath homogenization, centrifuge at 4℃, 8000rpm for 10min, take supernatant and placed on the ice for test.

2. Bacteria or cell: The ratio of bacteria/cell amount (10^6) : the volume of extract solution (mL) is 5~10:1(it is suggested to take about 5 million bacteria/cells and add 1 mL of extract solution). Bacteria/cell is split by ultrasonic (placed on ice, 200 W, work time 3 s, interval 10 s, total time 3min). Centrifuge at 8000g for 10 minutes at 4℃, take the supernatant and placed on the ice for test.

3. Serum (plasma) sample: Detect sample directly.If the solution is turbid, at 8000g for 10 minutes at 4℃, take the supernatant and placed on the ice for test.

II. Determination procedure

1. Preheat spectrophotometer/microplate reader for more than 30 minutes, adjust wavelength to 405 nm and set spectrophotometer zero with distilled water.
2. Operation table (Add the following reagents to 96 well plate or EP tube)

Reagent name (μL)	Test tube (T)	Control tube(C)	Blank tube (B)	Standard tube(S)
Sample	20	20	-	-
Reagent I	10	-	-	-
Distilled water	-	10	-	-
Reagent II	70	70	-	-
37℃ enzymatic reaction for 30min			-	-
Distilled water	-	-	100	-
Standard solution	-	-	-	100
Reagent III	100	100	100	100
After fully mixed, the absorbance value was measured at 405 nm, recorded as A_T and A_C, and $\Delta A_T = A_T - A_C$.			The absorbance value at 405 nm was directly measured after fully mixing, recorded as A_S and Calculate $\Delta A_S = A_S - A_B$.	

III. Calculation

(1) Calculate by sample protein concentration

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the generation of 1 μmol of p-Nitrophenol in the reaction system per hour every milligram protein.

β-GD activity (U/mg prot)=$\Delta A_T \div \Delta A_S \times C_S \times V_R \div (Cpr \times V_T) \div T \times F = \Delta A_T \div \Delta A_S \div Cpr \times F$

(2) Calculate by sample mass

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the generation of 1 μmol of p-Nitrophenol in the reaction system per hour every gram tissue.

β-GD activity (U/g mass)=$\Delta A_T \div \Delta A_S \times C_S \times V_R \div (W \div V_E \times V_T) \div T \times F = \Delta A_T \div \Delta A_S \div W \times F$

(3) Calculate by the number of bacteria or cells

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the generation of 1 μmol of p-Nitrophenol in the reaction system per hour every 10^6 cells or bacteria.

β-GD activity (U/10^6 cell)=$\Delta A_T \div \Delta A_S \times C_S \times V_R \div (W \div V_E \times V_T) \div T \times F = \Delta A_T \div \Delta A_S \div N \times F$

(4) Calculate by liquid volume

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the generation of 1 μmol of p-Nitrophenol in the reaction system per hour every milliliter serum (plasma).

β-GD activity (U/mL)=$\Delta A_T \div \Delta A_S \times C_S \times V_R \div V_T \div T \times F = \Delta A_T \div \Delta A_S \times F$

C_S：Standard tube p-Nitrophenol concentration，0.1μmol/mL；

V_R: Reaction system volume, 0.1mL;

Cpr: Sample protein concentration (mg/mL), additional measurements are required;

V_T: Sample volume , 0.02mL;

T: Reaction time, 0.5h;

V_E: Extract solution volume, 1mL;

W: Sample mass, g;

N: Total number of cells or bacteria, count by 10^6;

F: Sample dilution multiple:

Note:

1. Before the experiment, it is recommended to select 1-2 sample for pre-experiment. When A_T>1.5 the

References:

[1] Paul Awolade, Nosipho Cele, Nagaraju Kerru. Therapeutic significance of β-glucuronidase activity and its inhibitors: A review[J]. European Journal of Medicinal Chemistry, 2020, 187.

[2] Marta Dabek, Sheila I. McCrae, Valerie J. Stevens, Sylvia H. Duncan & Petra Louis. Distribution of b-glucosidase and b-glucuronidase activity and of b-glucuronidase gene gus in human colonic bacteria[J]. Fems Microbiology Ecology. 2008, 66(487–495).