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Aldolase (ALS) Activity Assay Kit

Product Number: BC2275

Shipping and Storage

Store at 2-8℃.

Component

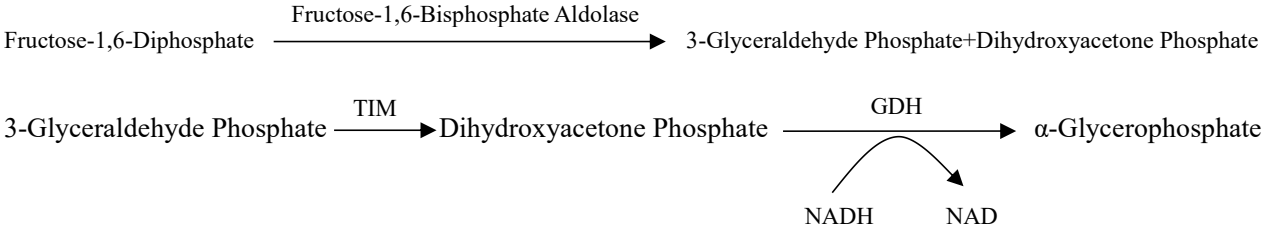
Component	100T
R1(Buffer/Substrate)	Powder × 5 bottles
R2(NADH)	Powder × 2 bottles
R3(GDH/TIM/LDH)	Liquid × 1 bottle

Solution preparation: R1: Add 20mL of distilled water to each bottle before use, and store at 2-8℃ for 2 weeks; R2: Dissolve 1mL of distilled water in each bottle before use and store at 2-8℃ for 4 weeks; R3: Can be used directly.

Description

Fructose 1,6-diphosphate aldolase (FBA) (EC. 4.1.2.13) is an important enzyme involved in the calvin cycle in glycolysis, gluconeogenesis, pentose phosphate pathway, and photosynthesis. It catalyzes the reversible cleavage of fructose 1,6-diphosphate into phosphodihydroxyacetone and 3-phosphoglyceraldehyde, and is widely present in animals, plants, and microorganisms, exhibiting different responses under various stress conditions.

Fructose 1,6-diphosphate aldolase catalyzes the conversion of fructose 1,6-diphosphate (F-1,6-DP) to 3-phosphoglyceraldehyde (GAP) and phosphodihydroxyacetone (DAP), and catalyzes the conversion of NADH and phosphodihydroxyacetone to NAD and α - phosphoglycerol under the action of phosphoglucose isomerase and α - phosphoglycerol dehydrogenase. The change in absorbance at 340nm can reflect the activity of fructose 1,6-diphosphate aldolase.



Note: It is recommended to select 2-3 samples with significant expected differences for pre experiments before the experiment.

Materials required but not supplied

UV spectrophotometer/biochemical analyzer, balance, low-temperature centrifuge, 1mL quartz colorimetric dish, adjustable pipette, mortar/homogenizer/cell ultrasonic disruptor, vortex shaker, ice and distilled water.

Sample processing

the sample size to be tested can be adjusted appropriately, and the specific proportion can be referred to in the literature.

1. Organization: According to the ratio of tissue mass (g) to extraction solution volume (mL) of 1:5-10 (it is recommended to weigh about 0.1g of tissue and add 1mL of extraction solution), homogenize thoroughly in an ice bath, then centrifuge at 8000g, 4℃ for 10 minutes, take the supernatant and place it on ice for testing.
2. Bacteria or cells: According to the ratio of the number of bacteria or cells (10^4) to the volume of extraction solution (mL) of 500-1000:1 (it is recommended to add 1mL of extraction solution to 5 million cells), the bacteria or cells are crushed by ice bath ultrasound (power 300w, ultrasound for 3 seconds, interval of 7 seconds, total time of 3 minutes); Then centrifuge at 8000g, 4℃ for 10 minutes, take the supernatant and place it on ice for testing.
3. Serum (plasma) samples: direct detection.

Protocol

1. Biochemical analyzer measurement:

1.1. Working fluid preparation: Prepare the working fluid according to the following proportions:

R1	R2	R3
2.5mL	0.05mL	0.01mL
5.0mL	0.1mL	0.02mL
10.0mL	0.2mL	0.04mL

1.2. Operation table:

	Sample tube	Blank tube	Calibration tube
Sample	35μL	-	-
Distilled water	-	35μL	-
Calibration solution	-	-	35μL
Working fluid	450μL	450μL	450μL

After thorough mixing, take a water bath at 37 ℃ for 5 minutes or react at 25 ℃ for 10 minutes.

2. Manual measurement:

2.1. Preheat the spectrophotometer for at least 30 minutes, adjust the wavelength to 340nm, and zero the distilled water.

2.2. Sample measurement:

	Sample blank tube	Sample determination tube	Calibration tube
Sample	0.2mL	0.2mL	-
Calibration serum	-	-	0.2mL
Distilled water	2.56mL	-	-
R1	-	2.5mL	2.5mL
R2	-	0.05mL	0.05mL
R3	-	0.01mL	0.01mL

After thorough mixing, take a 5-minute water bath at 37 ℃ and measure the absorbance values of the three tubes, denoted as A1. Continue the reaction at 37 ℃ for 20 minutes and measure the absorbance values of the three tubes, denoted as A2. Calculate ΔA=A1- A2.

FBA enzyme activity calculation

FBA enzyme activity=(Δ A sample determination tube - Δ A sample blank tube) ÷ Δ A calibration tube × calibration tube concentration.

Note

If Δ A is greater than 0.8, it is recommended to dilute the sample appropriately with the corresponding extraction solution before measurement, and multiply it by the dilution factor in the calculation formula.