Formaldehyde Dehydrogenase (FDH) Activity Assay Kit (Micromethod and liquid samples)

Note: Take two or three different samples for prediction before test.

Operation Equipment: Ultraviolet spectrophotometer/Microplate reader

Catalog Number: NA0198

Size: 100T/96S

Components:

Reagent	Size	Storage
Extract solution	Solution 110 mL×1	4℃
Reagent I	Solution 15 mL×1	4℃
Reagent II	Powder×2	-20℃
Reagent III	Powder×2	4℃
Reagent IV	Solution 2 mL×1	4℃

Solution preparation:

1. Reagent II: Add 0.25 mL distilled water before use. Unused regents should be store at -20℃ for two weeks. (One bottle of powder can be made 100T after dissolving. In order to prolong the use time, one more bottle of powder for this product)
2. Working solution of Reagent II: According to the amount required for the test, prepare the Working solution according to the ratio of Reagent II (μL): Distilled water (μL) =1:29, and prepare the reagents when it will be used. The Working solution of Reagent II should be used up on the same day if it is prepared on the same day.
3. Reagent III: Add 0.75 mL distilled water before use. Mix thoroughly. Unused reagents should be store at -20℃ for two weeks.

Product Description：

Formaldehyde dehydrogenase exists in most prokaryotes and all eukaryotes. It is an oxidoreductase that converts formaldehyde. Formaldehyde dehydrogenase can catalyze formaldehyde and NAD^+ to produce NADH. The absorbance at 340 nm will increase. By measuring the change at 340nm, the activity of formaldehyde dehydrogenase can be calculated.

Reagents and Equipment Required but Not Provided：

Ultraviolet spectrophotometer/microplate reader, desk centrifuge, pipette, micro quartz cuvette/96 well UV plate, mortar/homogenizer, ice and distilled water.

Procedure

I. Sample preparation:

Bacteria or cells: collect cells or bacteria to centrifuge and remove the supernatant. Suggested 5 million with 1mL of extract solution, split bacteria and cell with ultrasonication (power 30%, work time 3s,

interval 9s, for 3 min). centrifuge at 8000rpm and 4℃ for 10min, supernatant on ice is used for test. (If the supernatant is not clear enough, it is recommended to repeat the centrifugation steps above).
Serum and liquid samples: direct detection.

II. Determination procedure：

1. Preheat ultraviolet spectrophotometer/microplate reader for 30 minutes, adjust wavelength to 340 nm, set zero with distilled water.
2. Preheat the Reagent I and Reagent IV at 37℃ for 10 min.

Add 20 μL sample, 110 μL Reagent I, 50 μL Working solution of Reagent II, 10 μL Reagent III and 10 μL Reagent IV in the micro quartz cuvette or 96 well UV flat-bottom plate. Mix thoroughly. Measure the absorbance value A1 at 340 nm for 20s. Quickly put it into a water bath or incubator at 37℃ (mammal) or 25℃ (other species) for 5 min (the temperature can be adjusted to 37℃ or 25℃ with the temperature control function of the microplate reader). Take out and dry it quickly. Measure the absorbance value A2 for 5min20s. Calculation ΔA= A2-A1.

III. Calculations：

A. Calculated by micro-quartz cuvette

1. Calculate by number of bacteria or cells

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes production of 1 nmol of NADH in the reaction system per 10,000 bacteria or cells per minute.

FDH ($U/10^4$ cell) =ΔA÷ (ε×d) ×V_R ×10^9÷ (V_S×Cpr) ÷T×F=321.54×ΔA÷cells×F

2. Calculate by volume of serum (plasma) or liquid

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes production of 1 nmol of NADH in the reaction system per milliliter of serum (plasma) or liquid.

FDH (U/mL) =ΔA÷ (ε×d) ×V_R ×10^9÷ V_S ÷T×F=321.54×ΔA×F

3. Calculate by sample weight

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes production of 1 nmol of NADH in the reaction system per minute every gram tissue.

FDH (nmol/min/g weight) =ΔA÷ (ε×d) ×V_R×10^9÷ (V_S×W÷V_E) ÷T×F=321.54×ΔA÷W×F

V_S：Add sample volume,0.02 mL；

V_R: Total reaction volume, 0.0002L;

V_E: Extract solution volume, 1 mL;

ε：Micromolar extinction coefficient of NADH, 6220 L/mol/cm；

d：Optical path of cuvette, 1 cm；

T: Reaction time, 5 min;

Cpr: Protein concentration of sample, mg/mL;

W: Sample weight, g;

F: Dilution ratio.

B. Calculated by 96-well plate (UV plate)

Change d=1cm in the above formula to d=0.6, and then substitute the formula for calculation.

Note:

1. If the measured absorbance value A>1.0 or ΔA>0.5, it is recommended to dilute the sample before measuring, and multiply the dilution factor in the calculation formula; if the measured absorbance value is low or close to the blank OD value, it is recommended to increase the sample volume before performing the measurement.
2. Reagent IV is toxic. Please wear protective measures such as masks and gloves during the experiment.

Experimental example

1. Take 5 million Escherichia coli and add 1 mL extract, ultrasonically disrupt the cells (power 30%, ultrasonic 3s, interval 9s, total time 3min); then centrifuge at 8000 g, 4°C for 10 min, take the supernatant and follow the determination procedure. Measure by the micro quartz cuvette and calculate ΔA=A2-A1=0.2523-0.0396=0.2127, calculate the enzyme activity according to the number of bacteria:

FDH activity (U/10^4 cell)=321.54×ΔA÷cells =0.137 U/10^4 cell.

2. Take 0.02 mL bovine serum and operate according to the determination procedure, calculate ΔA=A2 - A1=0.1101-0.0772=0.0329, calculate the enzyme activity according to the liquid volume:

FDH activity (U/mL)= =321.54×ΔA=10.594 U/mL.

Related products

NA0203/NA0202 Formaldehyde Dehydrogenase (FDH) activity Assay Kit (Plant Samples)
NA0201/NA0200 Formaldehyde Dehydrogenase (FDH) activity Assay Kit (Animal Samples)