

Formaldehyde Dehydrogenase (FDH) Activity Assay Kit (Animal Samples)

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

Operation Equipment: Ultraviolet spectrophotometer

Catalog Number: AK0012

Size: 50T/48S

Components:

Reagent	Size	Storage
Extract solution	Solution 60 mL×1	4°C
Reagent I	Solution 35 mL×1	4°C
Reagent II	Powder×2	-20°C
Reagent III	Powder×2	4°C
Reagent IV	Solution 3 mL×1	4°C

Solution preparation:

1. Reagent II: Add 0.25 mL distilled water before use. Unused reagents should be store at -20°C for two weeks.
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 1.5 mL distilled water before use. Mix thoroughly. Unused reagents should be store at -20°C 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, desk centrifuge, pipette, 1 mL quartz cuvette, mortar/homogenizer, ice and distilled water.

Procedure

I. Sample preparation:

Tissue: According to the ratio of tissue weight (g): Extract solution (mL) = 1:5~10. It is suggested to weigh about 0.1 g of tissue and add 1 mL of Extract solution. Homogenize on ice. Centrifuge at 8 000 g, 4°C for 10 min. Take the supernatant for test.

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°C for 10 min.
3. Add 100μL sample, 550μL Reagent I, 250μL Working solution of Reagent II, 50μL Reagent III and 50μL Reagent IV in the 1 mL quartz cuvette. Mix thoroughly. Measure the absorbance value A1 at 340 nm for 20s. Quickly put it into a water bath or incubator at 37C for 5 min. Take out and dry it quickly. Measure the absorbance value A2 for 5min20s. Calculation $\Delta A = A_2 - A_1$.

III. Calculations :

1. Calculate by sample protein concentration

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 milligram protein.

$$\text{FDH (nmol/min/mg prot)} = \Delta A \div (\epsilon \times d) \times V_R \div (V_S \times \text{Cpr}) \times 10^9 \div T \times F = 321.54 \times \Delta A \div \text{Cpr} \times F$$

2. 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.

$$\text{FDH (nmol/min/g weight)} = \Delta A \div (\epsilon \times d) \times V_R \times 10^9 \div (V_S \times W \div V_E) \div T \times F = 321.54 \times \Delta A \div W \times F$$

V_S : Add sample volume, 0.1 mL;

V_R : Total reaction volume, 0.001L;

V_E : Extract solution volume, 1 mL;

ϵ : 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.

Note:

1. If the measured absorbance value $A > 1.0$ or $\Delta A > 0.5$, 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, it is recommended to increase the sample volume before performing the measurement.

Experimental example

1. Take 0.1 g mouse heart. Add 1 mL of Extract solution. Homogenize on ice. Centrifuge at 8 000 g, 4°C for 10 min. Take the supernatant for test. Following the measurement procedure. Calculate $\Delta A = A_2 - A_1 = 0.626 - 0.301 = 0.325$. Calculate the activity of formaldehyde dehydrogenase (FDH) in mouse heart according to the formula:

$$\text{FDH activity (nmol/min/g weight)} = 321.54 \times \Delta A \div W \times F = U/\text{g weight.}$$

2. Take 0.1 g mouse liver. Add 1 mL of Extract solution. Homogenize on ice. Centrifuge at 8 000 g, 4°C for 10 min. Take the supernatant and dilute ten times for test. Following the measurement procedure. Calculate $\Delta A = A_2 - A_1 = 0.224 - 0.136 = 0.088$. Calculate the activity of formaldehyde dehydrogenase (FDH)

in mouse heart according to the formula:

FDH activity (nmol/min/g weight) = $321.54 \times \Delta A \div W \times F = 2833.6$ U/g weight.

Related products

AK0014/AK0013 Formaldehyde Dehydrogenase (FDH) Activity Assay Kit (Plant Samples)

AK0010/AK0009 Formaldehyde Dehydrogenase (FDH) Activity Assay Kit (Micromethod and liquid samples)