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CheKine™ Micro Formaldehyde Dehydrogenase (FDH) Activity Assay Kit

Cat #: KTB3033 Size: 48 T/48 S 96 T/96 S

	Micro Formaldehyde Dehydrogenase (FDH) Activity Assay Kit		
REF	**Cat #:** KTB3033	LOT	**Lot #:** Refer to product label
	Applicable samples: Animal and Plant Tissues, Cells, Serum, Plasma or other Liquids		
	Storage: Stored at -20℃ for 6 months, protected from light		

Assay Principle

Formaldehyde is a non-specific reactive compound with proteins, nucleic acids and lipids, and is highly toxic to all organisms. As one of the family members of Zinc-containing medium chain alcohol dehydrogenase (ADH), formaldehyde dehydrogenase is widely present in prokaryotes and eukaryotes. This enzyme can use NAD^+ as a coenzyme to oxidize toxic formaldehyde and is a key enzyme in the formaldehyde oxidation pathway. FDH catalyzes formaldehyde and NAD^+ to produce NADH, and the absorbance value at 340 nm increases. The activity of FDH can be reflected by the change of absorbance value of NADH at 340 nm.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	48 T	96 T	
Extraction Buffer	60 mL	60×2 mL	4℃
Reagent Ⅰ	7.5 mL	15 mL	4℃
Reagent Ⅱ	Powder×1 vial	Powder×1 vial	-20℃
Reagent Ⅲ	Powder×1 vial	Powder×1 vial	4℃, protected from light
ReagentⅣ	0.75 mL	1.5 mL	4℃, protected from light

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

Materials Required but Not Supplied

- Microplate reader or ultraviolet spectrophotometer capable of measuring absorbance at 340 nm
- 96-well UV plate or microquartz cuvette, precision pipettes, disposable pipette tips
- Water bath, ice maker, centrifuge
- Deionized water
- Homogenizer (for tissue samples)

Reagent Preparation

Extraction Buffer: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃.

Reagent Ⅰ: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃.

Reagent Ⅱ: Prepare before use, 48 T add 3 mL deionized water, 96 T add 6 mL deionized water to dissolve it for use. The unused Reagent Ⅱ can be stored stored at -20℃ for one month after packaging.

Reagent Ⅲ: Prepare before use, 48 T add 0.75 mL deionized water, 96 T add 1.5 mL deionized water to dissolve it for use. The unused Reagent Ⅲ can be stored stored at 4℃ for one month, protected from light.

Reagent Ⅳ: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃, protected from light.

Note: Reagent Ⅳ has a pungent odor, so it is recommended to experiment in a fume hood.

Sample Preparation

Note: It is recommended to use fresh samples. If the experiment is not conducted immediately, the samples can be stored at -80℃ for 1 month. The temperature and time of thawing should be controlled during the determination. When thawing at room temperature, the sample should be thawed within 4 h.

1. Tissues: Weigh 0.1 g tissue , add 1 mL Extraction Buffer, homogenize on ice. Centrifuge at 10,000 g for 20 min at 4℃. Take the supernatant and place it on the ice for testing.
2. Cells: Collect 5×10^6 cells into the centrifuge tube, wash cells with cold PBS, discard the supernatant after centrifugation, add 1 mL Extraction Buffer, ultrasonically disrupt cells 3 min (power 30% or 300 W, ultrasonic 3 s, interval 7 s, total time for 3 min). Then centrifuge at 10,000 g for 10 min at 4℃. Take the supernatant and place it on the ice for testing.
3. Serum, Plasma and other Liquid Samples: Direct detection. If the solution has turbidity, centrifuge and take the supernatant for measurement.

Note: If the protein concentration of the sample is need to determined, it is recommended to use Abbkine catalog number: KTD3001 Protein Quantification Kit (BCA Assay) to measure the protein concentration of the sample.

Assay Procedure

1. Preheat the microplate reader or ultraviolet spectrophotometer for more than 30 min, and adjust the wavelength to 340 nm, ultraviolet spectrophotometer was returned to zero with deionized water.
2. Preheat the prepared Reagent Ⅱ at 37℃ for 5 min.
3. Sample measurement. (The following operations are operated in the 96-well UV plate or microquartz cuvette)

Regent	Test well (μL)
Sample	20
Reagent Ⅰ	110
Reagent Ⅱ	50
Reagent Ⅲ	10
Reagent Ⅳ	10
Mix well, record the absorbance values of 0 min and 30 min at 340 nm, mark as A1 and A2, and calculate $\Delta A=A_2-A_1$.	

Note: In order to guarantee the accuracy of experimental results, need to do a pre-experiment with 2-3 samples. If the number of samples is large, the reagents can be proportionally mixed into a working regent for use. If the ΔA is less than 0.01, the sample size can be appropriately increased or the reaction time can be appropriately extended (For example, the reaction time is 60 min). If the ΔA is greater than 1.0, the sample can be appropriately diluted with Extraction Buffer, the calculated result multiplied by the dilution factor.

Data Analysis

Note: We provide you with calculation formulae, including the derivation process and final formula. The two are exactly equal. It is suggested that the concise calculation formula in bold is final formula.

A. 96-well UV plates calculation formula

(1) Calculated by protein concentration:

Unit definition: One enzyme activity unit defines as 1 nmol NADH produced by 1 mg tissue proteins per min.

FDH (U/mg prot)=[ΔA×V_{Total}÷(ε×d)×10^9]÷(V_{Sample}×Cpr)÷T**=128.6×ΔA÷Cpr**

(2) Calculation according to the weight of the sample:

Unit definition: One enzyme activity unit defines as 1 nmol NADH produced by 1 g tissue per min

FDH (U/g fresh weight)=[ΔA×V_{Total}÷(ε×d)×10^9]÷(V_{Sample}×W÷$V_{Sample\ Total}$)÷T**=128.6×ΔA÷W**

(3) Calculation according to cell number

Unit definition: One enzyme activity unit defines as 1 nmol NADH produced by 10^4 cells per min

FDH (U/10^4 cell)=ΔA÷(ε×d)×V_{Tota}÷(V_{Sample}×500÷$V_{Sample\ Total}$)÷T**=128.6×ΔA÷500**

(4) Calculation according to the volume of liquid

Unit definition: One enzyme activity unit defines as 1 nmol NADH produced by 1 mL liquid per min

FDH (U/mL)=[ΔA×V_{Total}÷(ε×d)×10^9]÷V_{Sample}÷T**=128.6×ΔA**

Where: ε: NADH molar extinction coefficient, 6.22×10^3 L/mol/cm; d: 96-well UV plate diameter, 0.5 cm; V_{Total}: the total volume of the reaction system, 0.2 mL=2×10^{-4} L; V_{Sample}: the volume of the sample in the reaction system, 0.02 mL; $V_{Sample\ Total}$: The volume of Extraction Buffer added, 1 mL; Cpr: protein concentration (mg/mL); W: sample weight, g; T: reaction time, 30 min; 500: Total number of cells, 5×10^6.

B. Microquartz cuvette calculation formula

The optical diameter d:0.5 cm in the above calculation formula can be adjusted to d:1 cm for calculation.

Precautions

1. ReagentⅠⅤ is toxic, please wear protective measures such as mask and gloves during the experiment.

Typical Data

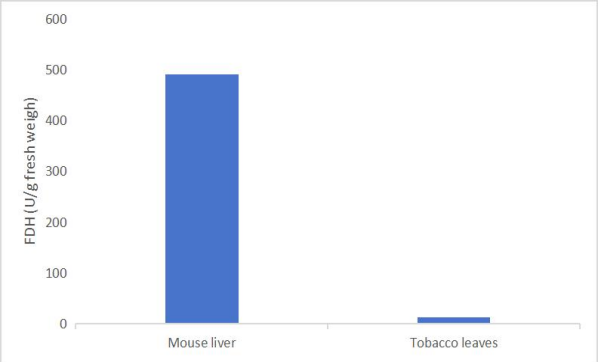

Figure 1. FDH activity in Mouse liver and Tobacco leaves was detected with this kit.

Recommended Products

Catalog No.	Product Name
KTB1015	CheKine™ Micro α-glucosidase(α-GC) Activity Assay Kit

KTB1121	CheKine™ Micro Pyruvate Acid (PA) Assay Kit

Disclaimer

The reagent is only used in the field of scientific research, not suitable for clinical diagnosis or other purposes. For your safety and health, please wear a lab coat and disposable gloves.