（FOR RESEARCH USE ONLY. DO NOT USE IT IN CLINICAL DIAGNOSIS !）

Catalog No：E-BC-F140

Specification：96T(40 samples)

Measuring instrument：Fluorescence Microplate Reader

(Ex/Em=340 nm/450 nm)

Detection range：0.008-2.5 U/L

Elabscience® Dihydroorotate Dehydrogenase (DHODH) Activity Fluorometric Assay Kit

This manual must be read attentively and completely before using this product. If you have any problem, please contact our Technical Service Center for help：

Toll-free: 1-888-852-8623

Tell: 1-832-243-6086

Fax: 1-832-243-6017

Email: techsupport@elabscience.com

Website: www.elabscience.com

Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

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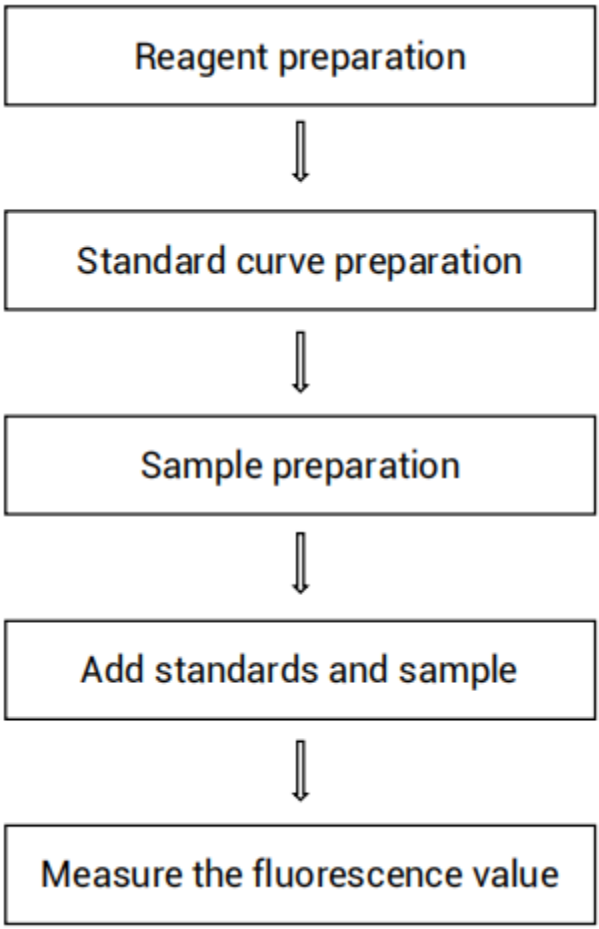
Assay summary



Intended use

This kit can be used to measure dihydroorotate dehydrogenase (DHODH) activity in animal tissue and cell samples.

Detection principle

Dihydroorotate Dehydrogenase (DHODH) is a key enzyme in the de novo synthesis pathway of pyrimidine nucleotides. DHODH catalyzes the formation of orotic acid and reacts with a chromogenic agent to produce a fluorescent substance. The fluorescence intensity is positively correlated with the activity of DHODH.

Kit components & storage

Item	Component	Size (96 T)	Storage
Reagent 1	Enzyme Reaction Solution	8 mL × 1 vial	-20°C, 12 months
Reagent 2	Cofactor	0.8 mL × 1 vial	-20°C, 12 months shading light
Reagent 3	Chromogenic Agent A	18 mL ×1 vial	-20°C, 12 months
Reagent 4	Chromogenic Agent B	22 mL×1 vial	-20°C, 12 months shading light
Reagent 5	Chromogenic Agent C	22 mL×1 vial	-20°C, 12 months shading light
Reagent 6	Chromogenic Agent D	4.4 mL×1 vial	-20°C, 12 months shading light
Reagent 7	10 mmol/L Standard	1 mL×1 vial	-20°C, 12 months shading light
	Black Microplate	96 wells	No requirement
	Plate Sealer	2 pieces	
	Sample Layout Sheet	1 piece	

Note: The reagents must be stored strictly according to the preservation conditions in the above table. The reagents in different kits cannot be mixed with each other. For a small volume of reagents, please centrifuge before use, so as not to obtain sufficient amount of reagents.

Materials prepared by users

Instruments:

Fluorescence microplate reader (Ex/Em=340 nm/450 nm), Incubator

Reagents:

PBS(0.01 M，pH 7.4)

Reagent preparation

① Equilibrate all reagents to 25°C before use.

② Cofactor will precipitate under low-temperature storage. After returning to 25°C, mix for 2 min using a vortex to restore its solubility.

③ The preparation of enzyme reaction working solution:
Before testing, please prepare sufficient enzyme reaction working solution. For example, prepare 100 μL of enzyme reaction working solution (mix well 90 μL of enzyme reaction solution and 10 μL of cofactor). The enzyme reaction working solution should be prepared on spot. Store at 25°C protected from light and used up within 4 h.

④ The preparation of 100 μmol/L standard:
Before testing, please prepare sufficient 100 μmol/L standard. For example, prepare 1000 μL of 100 μmol/L standard (mix well 10 μL of 10 mmol/L standard and 990 μL of PBS(0.01 M，pH 7.4)). The 100 μmol/L standard should be prepared on spot and used up within 8 h.

⑤ The preparation of standard curve:

Always prepare a fresh set of standards. Discard working standard dilutions after use.

Dilute 100 μmol/L standard solution with PBS(0.01 M，pH 7.4) to a serial concentration. The recommended dilution gradient is as follows: 0, 10, 20, 30, 40, 60, 80, 100 μmol/L. Reference is as follows:

Item	①	②	③	④	⑤	⑥	⑦	⑧
Concentration (μmol/L)	**0**	**10**	**20**	**30**	**40**	**60**	**80**	**100**
100 μmol/L Standard (μL)	0	20	40	60	80	120	160	200
PBS(0.01 M，pH 7.4)(μL)	200	180	160	140	120	80	40	0

Sample preparation

① Sample preparation

Tissue sample:

① Harvest the amount of tissue needed for each assay (initial recommendation 50 mg).

② Wash tissue in cold PBS (0.01 M, pH 7.4).

③ Homogenize 50 mg tissue in 950 μL PBS(0.01 M，pH 7.4)with a dounce homogenizer at 4°C.

④ Centrifuge at 12000×g for 10 min at 4°C to remove insoluble material. Collect supernatant and keep it on ice for detection. The supernatant should be used up within 8 h.

⑤ Meanwhile, determine the protein concentration of supernatant (E-BC-K318-M).

Cell samples:

① Harvest the number of cells needed for each assay (initial recommendation 1×10^6 cells).

② Wash tissue in cold PBS (0.01 M, pH 7.4).

③ Homogenize 1×10^6 cells in 1 mL PBS (0.01 M, pH 7.4) with a ultrasonic cell disruptor at 4°C.

④ Centrifuge at 12000×g for 10 min at 4°C to remove insoluble material. Collect supernatant and keep it on ice for detection. The supernatant should be used up within 8 h.

⑤ Meanwhile, determine the protein concentration of supernatant (E-BC-K318-M).

② Dilution of sample

The recommended dilution factor for different samples is as follows (for reference only):

Sample type	Dilution factor
5% Mouse liver tissue homogenate	1
5% Mouse kidney tissue homogenate	1
5% Mouse spleen tissue homogenate	1
1×10^6 Hela cells	1
1×10^6 Jurkat cells	1

Note: The diluent is PBS(0.01 M, pH 7.4). For the dilution of other sample types, please do pretest to confirm the dilution factor.

The key points of the assay

① During preparation, the enzyme reaction working solutions is prone to emulsion formation. Consequently, mixing should be performed quickly. If turbidity occur after preparation, the solution must be discarded and freshly prepared.

② Chromogenic agent A, chromogenic agent B, chromogenic agent C and chromogenic agent D must be added in sequence and cannot be added in any other order or mixed and then added to the system.

③ After the boiling water bath and centrifugation, transfer the supernatant to a new EP tube. Be careful to avoid absorbing sediment.

Operating steps

① Standard tube: add 30 μL of standard solution with different concentrations into the tubes.
Sample tube: add 30 μL of sample into the tubes.
Control tube: add 30 μL of sample into the tubes.
② Add 70 μL of enzyme reaction working solution into standard tubes and sample tubes.
③ Mix fully and incubate at 37°C for 40 min.
④ Add 70 μL of enzyme reaction working solution into control tubes.
⑤ Bath each tube in boiling water immediately. Centrifuge at 12000×g for 10 min.
⑥ Take 80 μL of supernatant from each tube and transfer to new EP tubes.
⑦ Add 160 μL of chromogenic agent A, 200 μL of chromogenic agent B, 200 μL of chromogenic agent C and 40 μL of chromogenic agent D in order into the new tubes. (**Note: The reagents must be added in the prescribed order)**.
⑧ Mix fully and chromogenic reaction for 4 min at 80°C accurately.
⑨ Cool down each tube with running water, centrifuge at 1000×g for 1 min. Take 200 μL of supernatant into the microplate.
⑩ Measure the fluorescence at the excitation wavelength of 340 nm and the emission wavelength of 450 nm, recorded as F.

Calculation

The standard curve:

1. Average the duplicate reading for each standard.

2. Subtract the mean fluorescence value of the blank (Standard #①) from all standard readings. This is the absoluted fluorescence value.

3. Plot the standard curve by using absoluted fluorescence value of standard and correspondent concentration as y-axis and x-axis respectively. Create the standard curve (**y = ax + b**) with graph software (or EXCEL).

The sample:

1. Animal tissue or cell samples:

Definition: The amount of enzyme in 1 g tissue or cell protein per 1 min that produce 1 μmol of orotic acid at 37°C is defined as 1 unit.

$$\text{DHODH activity (U/gprot)} = \frac{\Delta F - b}{a} \div C_{pr} \div T \times f$$

2. Animal tissue samples:

Definition: The amount of enzyme in 1 g tissue per 1 min that produce 1 μmol of orotic acid n at 37°C is defined as 1 unit.

$$\text{DHODH activity (U/g wet weight)} = \frac{\Delta F - b}{a} \div (m \times \frac{V_1}{V_2}) \div T \times f$$

[Note]

ΔF: $F_{sample} - F_{control}$.

V_1: The volume of sample, μL.

V_2: The volume of PBS(0.01 M,pH 7.4) added when homogenizing tissue sample, μL.

m: The weight of tissue, g.

T: Reaction time, 40 min.

f: Dilution factor of sample before tested.

C_{pr}: Concentration of protein in sample, gprot/L.

Appendix I Performance Characteristics

1. Parameter:

Intra-assay Precision

Three mouse liver tissue homogenate samples were assayed in replicates of 20 to determine precision within an assay. (CV = Coefficient of Variation)

Parameters	Sample 1	Sample 2	Sample 3
Mean (U/L)	0.5	1.0	2.0
%CV	5.9	2.2	5.9

Inter-assay Precision

Three mouse liver tissue homogenate samples were assayed 20 times in duplicate by three operators to determine precision between assays.

Parameters	Sample 1	Sample 2	Sample 3
Mean (U/L)	0.5	1.0	2.0
%CV	6.6	8.6	7.8

Recovery

Take three samples of high concentration, middle concentration and low concentration to test the samples of each concentration for 6 times parallelly to get the average recovery rate of 99.3%.

	Sample 1	Sample 2	Sample 3
Expected Conc(U/L)	0.5	1.0	2.0
Observed Conc(U/L)	0.48	1.01	2.04
Recovery rate (%)	95	101	102

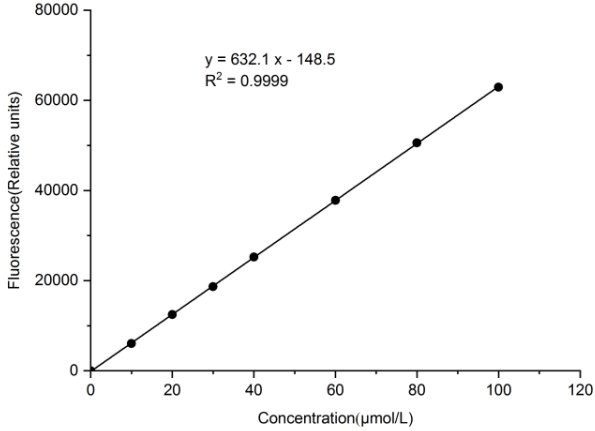
Sensitivity

The analytical sensitivity of the assay is 0.008 U/L. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 20 times, and calculating the corresponding concentration.

2. Standard curve:

As the fluorescence value of the standard curve may vary according to the conditions of the actual assay performance (e.g. operator, pipetting technique or temperature effects), so the standard curve and data are provided as below for reference only：

Concentration (μmol/L)	0	10	20	30	40	60	80	100
F value	2316	8376	14497	20699	27214	39894	53156	64548
	2320	8308	15108	21245	27858	40408	52677	65934
Average F value	2318	8342	14803	20972	27536	40151	52917	65241
Absoluted F value	0	6024	12485	18654	25218	37833	50599	62923



Appendix Π Example Analysis

Example analysis：

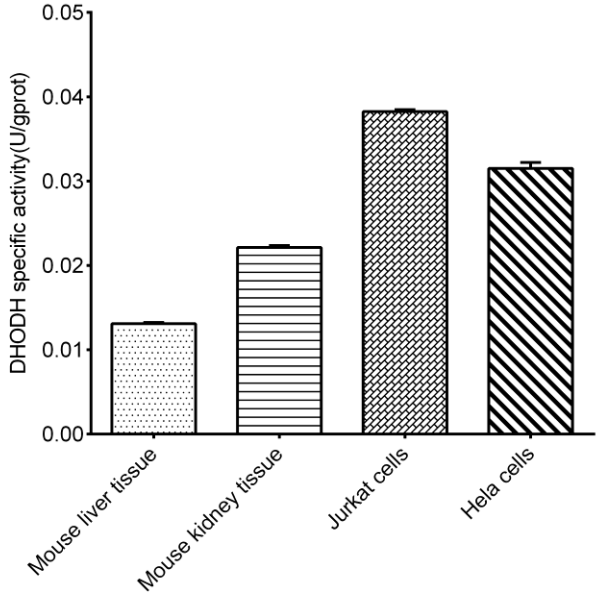
Take 30 μL of 5% mouse liver tissue homogenate and carry the assay according to the operation steps. The results are as follows:

Standard curve: y = 632.1 x - 148.5, the F value of the control well is 5570, the F value of the sample well is 7627.5, the concentration of protein in sample is 6.48 gprot/L). The calculation result is:

DHODH activity (U/gprot) = (7627.5 - 5570 + 148.5) ÷ 632.1 ÷ 6.48 ÷ 40

=0.013 U/gprot

Detect 5% mouse liver tissue homogenate (the concentration of protein is 6.48 gprot/L), 5% mouse kidney tissue homogenate (the concentration of protein is 4.21 gprot/L), 1×10^6 Jurkat cells (the concentration of protein is 0.71 gprot/L) and 1×10^6 Hela cells (the concentration of protein is 0.55 gprot/L) according to the protocol, the result is as follows:



Statement

1. This assay kit is for Research Use Only. We will not response for any arising problems or legal responsibilities causing by using the kit for clinical diagnosis or other purpose.
2. Please read the instructions carefully and adjust the instruments before the experiments. Please follow the instructions strictly during the experiments.
3. Protection methods must be taken by wearing lab coat and latex gloves.
4. If the concentration of substance is not within the detection range exactly, an extra dilution or concentration should be taken for the sample.
5. It is recommended to take a pre-test if your sample is not listed in the instruction book.
6. The experimental results are closely related to the situation of reagents, operations, environment and so on. Elabscience will guarantee the quality of the kits only, and NOT be responsible for the sample consumption caused by using the assay kits. It is better to calculate the possible usage of sample and reserve sufficient samples before use.