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

Catalog No：E-BC-K816-M

Specification：48T (23 samples)/96T (47 samples)

Measuring instrument：Microplate reader(340 nm)

Detection range：0.02-400 U/L

Elabscience® Dihydrofolate Reductase (DHFR) Activity Colorimetric 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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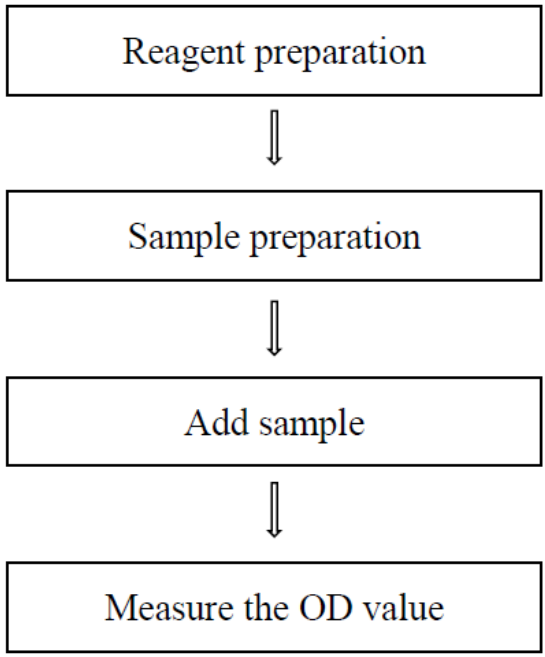
Assay summary



Intended use

This kit can be used to measure dihydrofolate reductase (DHFR) activity in serum, plasma, cell, animal and plant tissues samples.

Detection principle

Dihydrofolate Reductase (DHFR) is one of the key enzymes in nucleic acid metabolism and the main target enzyme in cancer drug therapy, and has been widely studied in recent years.

The detection principle of this kit is as follows: DHFR catalyzes substrate reactions to consume NADPH. NADPH has a characteristic absorption peak at 340 nm. The activity of DHFR can be characterized by measuring the rate of decrease in absorbance at 340 nm.

Kit components & storage

Item	Component	Size 1(48 T)	Size 2(96 T)	Storage
Reagent 1	Extraction Solution	25 mL × 1 vial	50 mL × 1 vial	-20℃, 12 months
Reagent 2	Substrate Stock Solution	2 mL × 1 vial	4 mL × 1 vial	-20℃, 12 months, shading light
Reagent 3	Buffer Solution	14 mL × 1 vial	28 mL × 1 vial	-20℃, 12 months, shading light
Reagent 4	Substrate A	Powder × 1 vial	Powder × 2 vials	-20℃, 12 months, shading light
Reagent 5	Substrate B	Powder × 1 vial	Powder × 2 vials	-20℃, 12 months, shading light
Reagent 6	Matrix Solution	0.9 mL × 1 vial	1.8 mL × 1 vial	-20℃, 12 months, shading light
	UV-Microplate	96 wells		No requirement
	Plate Sealer	2 pieces		

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:

Microplate reader (optimum wavelength: 340 nm), Incubator

Reagent preparation

① Equilibrate all the reagents to 25°C before use.

② The preparation of substrate A working solution:
Dissolve one vial of substrate A with 1 mL of substrate stock solution, mix well to dissolve. Keep it on ice during use. Aliquoted storage at -20°C for 7 days protected from light.

③ The preparation of substrate working solution:
Before testing, please prepare sufficient substrate working solution according to the test wells. For example, prepare 23 μL of substrate working solution (mix well 5 μL of substrate A working solution and 18 μL of buffer solution). The substrate working solution should be prepared on spot. Store at 2-8°C for 8 h protected from light.

④ The preparation of substrate B working solution:
Dissolve one vial of substrate B with 1.4 mL of double distilled water, mix well to dissolve. Aliquoted storage at -20°C for 7 days protected from light.

⑤ The preparation of reaction working solution:

For each well, prepare 140 μL of reaction working solution (mix well 14 μL of matrix solution and 126 μL of buffer solution). The reaction working solution should be prepared on spot. Store at 2-8°C for 8 h protected from light.

Sample preparation

① Sample preparation

Serum or plasma samples: detect directly.

Tissue sample:

① Harvest the amount of tissue needed for each assay (initial recommendation 20 mg).

② Homogenize 20 mg tissue in 180 μL extraction solution with a dounce homogenizer at 4°C.

③ Centrifuge at 10000×g for 10 min at 4°C to remove insoluble material. Collect supernatant and keep it on ice for detection. The supernatant should be detected within 5 h.

Cell samples:

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

② Homogenize 1×10^6 cells in 200 μL extraction solution with a ultrasonic cell disruptor at 4°C.

③ Centrifuge at 10000×g for 10 min at 4°C to remove insoluble material. Collect supernatant and keep it on ice for detection. The supernatant should be detected within 5 h.

② **Dilution of sample**

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

Sample type	Dilution factor
Rat serum	1
10% Rat liver tissue homogenate	1
10% Mouse spleen tissue homogenate	1
10% Mouse kidney tissue homogenate	1
10% Mouse lung tissue homogenate	1
10% Scindapsus aureus leaf tissue homogenate	1
1×10^6 Hela cells	1

Note: The diluent is extraction solution. For the dilution of other sample types, please do pretest to confirm the dilution factor.

Operating steps

① Blank well: Add 20 μL of double distilled water into wells.
Sample well: Add 20 μL of samples into wells.
Control well: Add 20 μL of samples into wells.

② Add 20 μL of substrate working solution into blank and sample wells.

③ Add 20 μL of substrate B working solution into each well.

④ Add 140 μL of reaction working solution into blank and sample wells.
Add 160 μL of reaction working solution into control well.

⑤ Mix fully for 5 s with microplate reader and measure the OD value of each well at 340 nm, as A_1.

⑥ Incubate at 37°C for 25 min protected from light and measure the OD value of each well at 340 nm, as A_2.

Calculation

The sample:

1. Serum (plasma) sample:

Definition: The amount of enzyme in 1 L serum or plasma per 1 min that catalyze 1 μmol NADPH at 37 °C is defined as 1 unit.

$$\text{DHFR activity (U/L)} = (\Delta A_1 - \Delta A_2 - \Delta A_3) \times V_1 \times f \div \varepsilon \div d \div V_2 \div T \times 10\text{^}6^*$$

2. Tissue sample:

Definition: The amount of enzyme in 1 kg tissue per 1 min that catalyze 1 μmol NADPH at 37 °C is defined as 1 unit.

$$\text{DHFR activity (U/kg wet weight)} = (\Delta A_1 - \Delta A_2 - \Delta A_3) \times V_1 \times f \div \varepsilon \div d \div V_2 \div T \div \frac{m}{V} \times 10\text{^}6^*$$

3. Cell sample:

Definition: The amount of enzyme in 1×10^6 cells samples per 1 min that catalyze 1 μmol NADPH at 37 °C is defined as 1 unit.

$$\text{DHFR activity (U/10^9)} = (\Delta A_1 - \Delta A_2 - \Delta A_3) \times V_1 \times f \div \varepsilon \div d \div V_2 \div T \div \frac{n}{V} \times 10\text{^}6^*$$

[Note]

ΔA_1: The change OD value of the sample well, $\Delta A_1 = A_1 - A_2$.

ΔA_2: The change OD value of the control well, $\Delta A_2 = A_1 - A_2$.

ΔA_3: The change OD value of the blank well, $\Delta A_3 = A_1 - A_2$.

f: Dilution factor of sample before test.

V_1: The volume of reaction system, 0.2 mL.

V_2: The volume of sample added to the reaction system, 0.02 mL.

T: Reaction time, 25 min.

ε: The molar extinction coefficient of chromogenic substance at 340 nm, 6.22 × 10^3 $L \cdot mol^{-1} \cdot cm^{-1}$.

d: Optical path, 0.6 cm.

m: The wet weight of sample, g.

n: The number of cell sample/10^6.

V: The volume of extraction solution in the preparation step, mL.

10^6* : 1 mol/L = 1000000 μmol/L

Appendix I Performance Characteristics

1. Parameter:

Intra-assay Precision

Three rat serum 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）	50	100	250
%CV	2.5	3.0	3.6

Inter-assay Precision

Three rat serum were assayed 20 times in duplicate by three operators to determine precision between assays.

Parameters	Sample 1	Sample 2	Sample 3
Mean (U/L)	50	100	250
%CV	5.0	7.2	6.6

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%.

	Sample 1	Sample 2	Sample 3
Expected Conc. (UL)	50	100	250
Observed Conc. (U/L)	49.0	99	250
Recovery rate (%)	98.0	99.0	100.0

Sensitivity

The analytical sensitivity of the assay is 0.02 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.

Appendix Π Example Analysis

Example analysis：

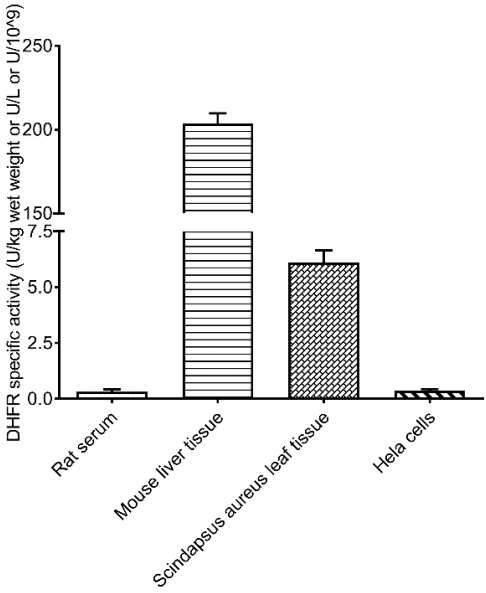
Take 20 μL of 10% mouse liver tissue homogenate and carry the assay according to the operation steps. The results are as follows:

The A_1 of the sample well is 1.437, the A_2 of the sample well is 1.013, ΔA_1 = 1.437 – 1.013 = 0.424, The A_1 of the control well is 1.250, the A_2 of the control well is 1.037, ΔA_2 = 1.250 – 1.037 = 0.213. The A_1 of the blank well is 0.902, the A_2 of the blank well is 0.878, ΔA_3 = 0.902 – 0.878 = 0.024, and the calculation result is:

$$\text{DHFR activity (U/kg wet weight)} = (0.424 - 0.213 - 0.024) \times 0.2 \div 6220 \div 0.6 \div 0.02 \div 25 \div \frac{0.1}{0.9} \times 10\text{^}6^*$$

$$= 180.39 \text{ U/Kg wet weight}$$

Detect rat serum, 10% mouse liver tissue homogenate, 10% scindapsus aureus leaf tissue homogenate and 1×10^6 Hela cells, 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.