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

Catalog No：E-BC-K908-M

Specification：96T (40 samples)

Measuring instrument：Microplate reader (445-465 nm)

Detection range：0.696-33.106 U/L

Elabscience® Fumarase (FUM) 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
Tel: 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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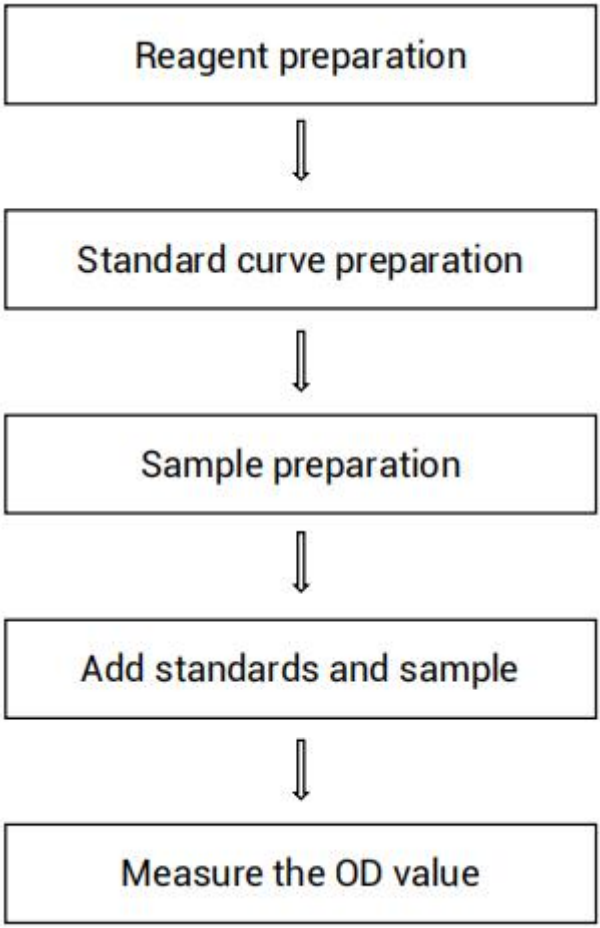
Assay summary



Intended use

This kit can be used to measure fumarase (FUM) activity in serum (plasma), animal tissues and cell samples.

Detection principle

Fumarase (FUM), also known as malate lyase, fumarate hydrase or fumarase, is an important enzyme in the tricarboxylic acid cycle, which can catalyze the hydration of fumarase to synthesize malic acid. FUM is a kind of intracellular enzyme that exists widely in plants and animals.

The detection principle of this kit: fumarase catalyze fumaric acid products under the action of electronic coupler to reduce the chromogenic reagent to orange products, detection at about 450 nm has the maximum absorption peak.

Kit components & storage

Item	Component	Size (96 T)	Storage
Reagent 1	Extraction Solution	50 mL × 2 vials	-20°C, 12months shading light
Reagent 2	Buffer	50 mL × 1 vial	-20°C, 12months shading light
Reagent 3	Substrate	0.3 mL × 1 vial	-20°C, 12months shading light
Reagent 4	Oxidant Reagent	Powder × 2 vials	-20°C, 12months shading light
Reagent 5	Catalyst	0.3 mL × 1 vial	-20°C, 12months shading light
Reagent 6	Chromogenic Agent	1.5 mL × 2 vials	-20°C, 12months shading light
Reagent 7	0.5 mmol/L Standard Solution	6 mL × 1 vial	-20°C, 12months shading light
	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:

Microplate reader (445-465 nm, optimum wavelength: 450 nm), Incubator (37℃)

Reagents:

PBS (0.01 M, pH 7.4)

Reagent preparation

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

② Preparation of substrate working solution：

Before testing, please prepare sufficient substrate working solution according to the test wells. For example, prepare 100 μL of substrate working solution (mix well 95 μL of extraction solution and 5 μL of substrate). The substrate working solution should be prepared on spot protected from light and used up the same day.

③ Preparation of oxidant working solution：

Dissolve one vial of oxidant reagent with 1 mL of double distilled water, mix well to dissolve. Store at -20°C for 7 days protected from light.

④ Preparation of measuring working solution：

Before testing, please prepare sufficient measuring working solution according to the test wells. For example, prepare 500 μL of measuring working solution (mix well 440 μL of buffer, 50 μL of oxidant working solution and 10 μL of catalyst). The measuring working solution

should be prepared on spot.

⑤ The preparation of standard curve：

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

Dilute 0.5 mmol/L standard solution with double distilled water to a serial concentration. The recommended dilution gradient is as follows: 0, 0.10, 0.15, 0.20, 0.30, 0.35, 0.40, 0.50 mmol/L. Reference is as follows:

Item	①	②	③	④	⑤	⑥	⑦	⑧
Standard (mmol/L)	**0**	**0.10**	**0.15**	**0.20**	**0.30**	**0.35**	**0.40**	**0.50**
0.5 mmol/L Standard(μL)	0	40	60	80	120	140	160	200
Double distilled water (μL)	200	160	140	120	80	60	40	0

Sample preparation

① Sample preparation

Serum and plasma: detect directly. If not detected on the same day, the serum or plasma can be stored at -80°C for a month.

Tissue sample:

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

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

③ Homogenize 20 mg tissue in 180 μL extraction solution with a dounce homogenizer at 4°C.

④ Centrifuge at 10000×g for 10 min at 4°C to remove insoluble material. Collect supernatant and keep it on ice for detection.

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

Cell (adherent or suspension) samples:

① Harvest the number of cells needed for each assay (initial

recommendation 1×10^6 cells).

② Wash cells with PBS (0.01 M, pH 7.4).

③ Homogenize 1×10^6 cells in 200 μL extraction solution with a ultrasonic cell disruptor at 4°C.

④ Centrifuge at 10000×g for 10 min at 4°C to remove insoluble material. Collect supernatant and keep it on ice for detection.

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
10% Mouse liver tissue homogenization	200-300
10% Mouse kidney tissue homogenization	200-300
10% Mouse heart tissue homogenization	200-300
10% Mouse lung tissue homogenization	200-300
Rat plasma	2-4
Mouse serum	2-4
Mouse plasma	2-4
Human serum	2-4
1×10^6 293T cell	2-4
1×10^6 Hela cell	2-4

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

Operating steps

① Standard well: add 20 μL of standards with different concentrations into standard wells.
Sample well: add 20 μL of sample into sample wells.
Control well: add 20 μL of sample into control wells.

② Add 40 μL of substrate working solution into standard wells and sample wells, add 40 μL of extraction solution into control wells.

③ Mix fully with microplate reader for 5 s and incubate at 37°C for 5 min protected from light.

④ Add 120 μL of measuring working solution to each well.

⑤ Add 20 μL of chromogenic agent to each well.

⑥ Mix fully with microplate reader for 5 s and incubate at 37°C for 15 min. Measure the OD values of each well with microplate reader at 450 nm.

Calculation

The standard curve:

1. Average the duplicate reading for each standard.

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

3. Plot the standard curve by using absoluted OD 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. Serum (plasma) samples:

Definition: The amount of enzyme in 1 L of serum (plasma) that catalyze the substrate to produce 1 μmol production at 37°C for 1 min is defined as 1 unit.

$$\text{FUM activity (U/L)} = (\Delta A_{450} - b) \div a \div T \times f \times 1000$$

2. Tissue and cell samples:

Definition: The amount of enzyme in 1 g of tissue or cell protein that catalyze the substrate to produce 1 μmol production at 37°C for 1 min is defined as 1 unit.

$$\text{FUM activity (U/gprot)} = (\Delta A_{450} - b) \div a \div T \times f \div C_{pr} \times 1000$$

[Note]

ΔA_{450}: The absoluted OD value of sample, $\Delta A_{450} = A_{sample} - A_{control}$.

f: Dilution factor of sample before test.

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

1000: 1 mmol/L=1000 μmol/L.

Appendix I Performance Characteristics

1. Parameter:

Intra-assay Precision

Three mouse serum 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)	10.00	20.00	30.00
%CV	2.4	3.0	5.0

Inter-assay Precision

Three mouse serum 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)	10.00	20.00	30.00
%CV	5.8	6.5	7.7

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 96.3%.

	Sample 1	Sample 2	Sample 3
Expected Conc (U/L)	10	20	30
Observed Conc(U/L)	9.2	19.0	30.6
Recovery rate (%)	92	95	102

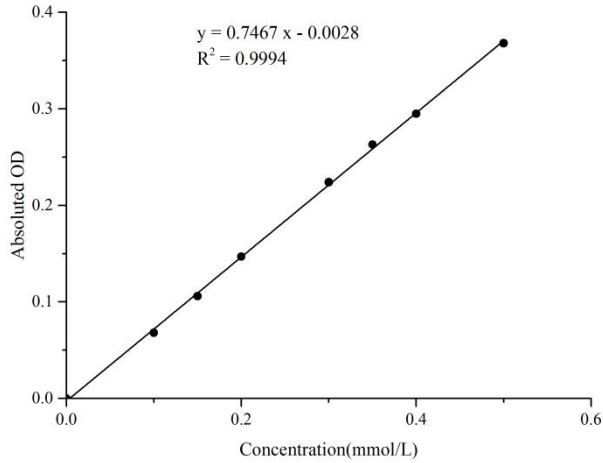
Sensitivity

The analytical sensitivity of the assay is 0.696 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 OD 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 (mmol/mL)	**0**	**0.10**	**0.15**	**0.20**	**0.30**	**0.35**	**0.40**	**0.50**
OD	0.111	0.190	0.225	0.262	0.342	0.374	0.412	0.486
	0.113	0.181	0.219	0.260	0.337	0.376	0.408	0.481
Average OD	0.112	0.186	0.222	0.261	0.340	0.375	0.410	0.484
Absoluted OD	0	0.074	0.110	0.149	0.228	0.263	0.298	0.368



Appendix Π Example Analysis

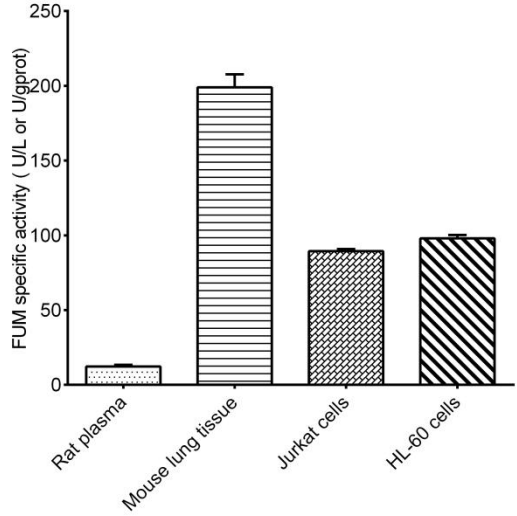
Example analysis：

Take 20 μL of 10% mouse lung tissue homogenization (dilute for 250 times) into the well, and carry the assay according to the operation steps. The results are as follows:

standard curve: y = 0.7467 x - 0.0028, the average OD value of the sample is 0.155, the average OD value of the control is 0.098, ΔA_{450}= A_{sample} - $A_{control}$ = 0.155 - 0.098 = 0.057, the concentration of protein in sample is 6.82 gprot/L L, and the result is:

FUM activity(U/gprot) = (0.057 + 0.0028)÷ 0.7467 ÷ 15 × 250 ÷ 6.82 × 1000 = 195.71 U/gprot

Detect rat plasma (dilute for 2 times), 10% mouse lung tissue homogenization (the concentration of protein is 6.82 gprot/L, dilute for 250 times), 1×10^6 Jurkat cells (the concentration of protein is 0.855 gprot/L, dilute for 3 times), 1×10^6 HL-60 cells (the concentration of protein is 0.956 gprot/L, dilute for 3 times) 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.