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

Catalog No：E-BC-K793-M

Specification：96T(40 samples)

Measuring instrument：Microplate reader(390-410 nm)

Detection range：1.05-39.7 U/L

Elabscience® Monoacylglycerol Lipase (MAGL) 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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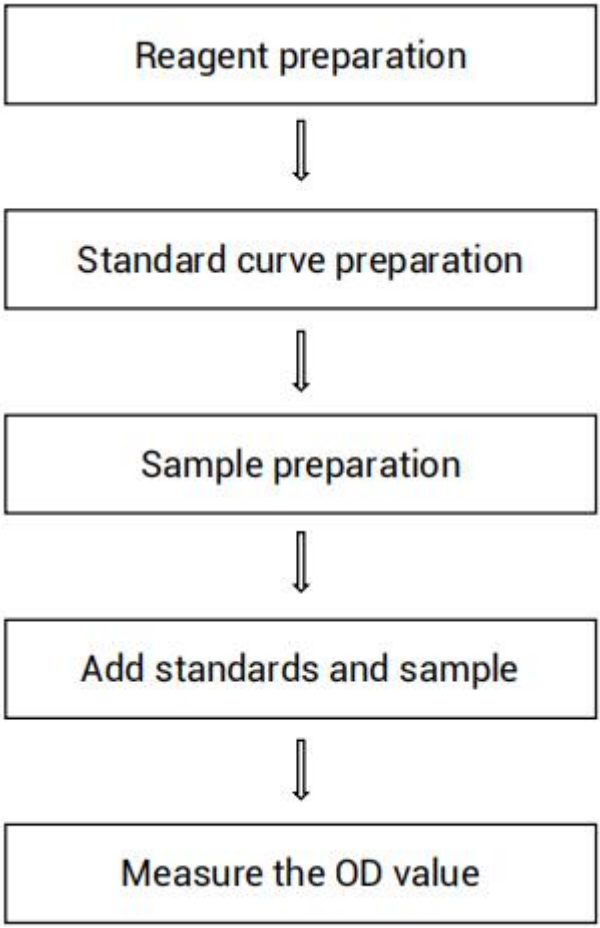
Assay summary



Intended use

This kit can measure monoacylglycerol lipase (MAGL) activity in serum (plasma), animal tissue and cell samples.

Detection principle

Monoacylglycerol lipase (MAGL) is a widely distributed serine hydrolase. MAGL can not only decompose triacylglycerol, which plays an important role in lipid metabolism, but also hydrolyze 2-arachidonic acid and regulate the signaling of the cannabinoid system in the body. The principle of this kit is that the substrate is catalyzed by MAGL, and the product generated can react with the chromogenic agent. By measuring the amount of chromogenic substance produced per unit time, the MAGL enzyme activity in the sample can be calculated.

Kit components & storage

Item	Component	Size (96 T)	Storage
Reagent 1	Buffer Solution	25 mL × 1 vial	-20°C, 12 months
Reagent 2	Substrate	1.2 mL × 1 vial	-20°C, 12 months, shading light
Reagent 3	Inhibitor	0.5 mL × 1 vial	-20°C, 12 months, shading light
Reagent 4	Lysis Solution	5 mL× 1 vial	-20°C, 12 months
Reagent 5	1 mmol/L Standard	2 mL × 1 vial	-20°C, 12 months, 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 (390-410 nm, optimum wavelength: 400 nm)

Reagents:

Normal saline (0.9% NaCl)

Reagent preparation

① Equilibrate all reagents to 25℃ protected from light before use.

② The preparation of working solution：

For each well, prepare 180 μL of working solution (mix well 9 μL of substrate and 171 μL of buffer solution). The working solution should be prepared on spot protected from light and used up within 30 min.

③ The preparation of standard curve：

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

Dilute 1 mmol/L standard with double distilled water to a serial concentration. The recommended dilution gradient is as follows: 0, 0.2, 0.4, 0.5, 0.6, 0.7, 0.8, 1 mmol/L. Reference is as follows:

Item	①	②	③	④	⑤	⑥	⑦	⑧
Concentration (mmol/L)	**0**	**0.2**	**0.4**	**0.5**	**0.6**	**0.7**	**0.8**	**1**
1 mmol/L Standard (μL)	0	40	80	100	120	140	160	200
Double distilled water (μL)	200	160	120	100	80	60	40	0

Sample preparation

① Sample preparation

Plasma or serum samples: Test directly, samples can be stored at -80ºC 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 normal saline (0.9% NaCl) with a dounce homogenizer at 4℃.

④ Centrifuge at 10000×g for 10 min at 4℃ 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 Lyse 1×10^6 cells with 200 μL lysis solution. Place on the ice box and mix well every 5 min, lyse for 15 min.

④ Centrifuge at 10000×g for 10 min at 4℃ 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 kidney tissue homogenate	50-500
10% Mouse heart tissue homogenate	3-10
10% Mouse liver tissue homogenate	50-500
10% Mouse lung tissue homogenate	20-200
1×10^6 K562 cells	1
1×10^6 Molt cells	1
Mouse plasma	3-50
Fetal bovine serum	5-50

Note: The diluent is normal saline (0.9% NaCl). For the dilution of other sample types, please do pretest to confirm the dilution factor.

The key points of the assay

The standard and working solution should be used and prepared protected from light. The working solution should be used up within 30 min.

Operating steps

① Standard well: Add 20 μL of standard solution with different concentrations into the corresponding well.
Sample well: Add 20 μL of sample into sample well.
Control well: Add 20 μL of sample into control well.

② Add 10 μL of inhibitor into control well.
Add 10 μL of normal saline (0.9% NaCl) into sample well.

③ Incubate at 37℃ for 10 min protected from light.

④ Add 190 μL of buffer solution into standard well.
Add 180 μL of working solution into sample and control well.

⑤ Incubate at 37 ℃ for 5 min protected from light. Measure the OD values of each well at 400 nm with microplate reader.

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 serum sample per 1 min that catalytic the substrate to produce 1 μmol *p*-nitrophenol at 37 °C is defined as 1 unit.

$$\text{MAGL activity (U/L)} = (\Delta A - b) \div a \div t \times f \times 1000$$

2. Tissue and cell samples:

Definition: The amount of enzyme in 1 g sample protein per 1 min that catalytic the substrate to produce 1 μmol *p*-nitrophenol at 37°C is defined as 1 unit.

$$\text{MAGL activity (U/gprot)} = (\Delta A - b) \div a \div t \div C_{pr} \times f \times 1000$$

[Note]

ΔA: $\Delta A = A_{Sample} - A_{Control}$.

T: the time of tissue sample reaction, 5 min.

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

f: Dilution factor of sample before test.

1000：1 mmol/L=1000 μmol/L.

Appendix I Performance Characteristics

1. Parameter:

Intra-assay Precision

Three mouse plasma 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)	5.40	21.00	34.00
%CV	1.8	5.0	2.6

Inter-assay Precision

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

Parameters	Sample 1	Sample 2	Sample 3
Mean (U/L)	5.40	21.00	34.00
%CV	3.3	10.2	8.7

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 97.5%.

	Sample 1	Sample 2	Sample 3
Expected Conc.（U/L）	5.40	21.00	34.00
Observed Conc.（U/L）	5.13	21	33.15
recovery rate(%)	95	100	97.5

Sensitivity

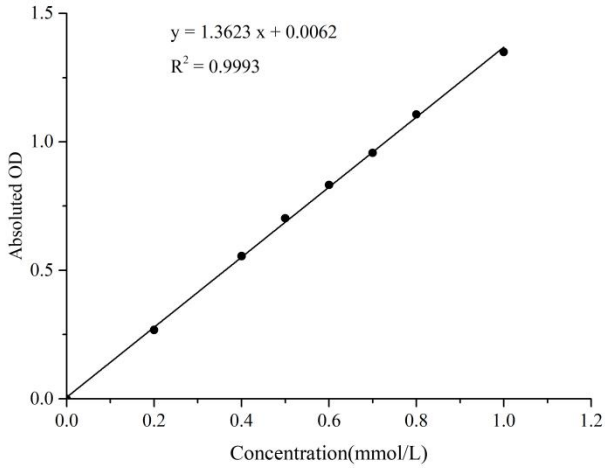
The analytical sensitivity of the assay is 1.05 U/L. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 20 times, and calculating the corresponding

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/L)	0	0.2	0.4	0.5	0.6	0.7	0.8	1
OD Value	0.041	0.305	0.597	0.742	0.869	0.995	1.148	1.36
	0.041	0.311	0.593	0.744	0.877	1.001	1.148	1.42
Average OD	0.041	0.308	0.596	0.743	0.873	0.998	1.148	1.39
Absoluted OD	0	0.267	0.555	0.702	0.832	0.957	1.107	1.348



Appendix Π Example Analysis

Example analysis：

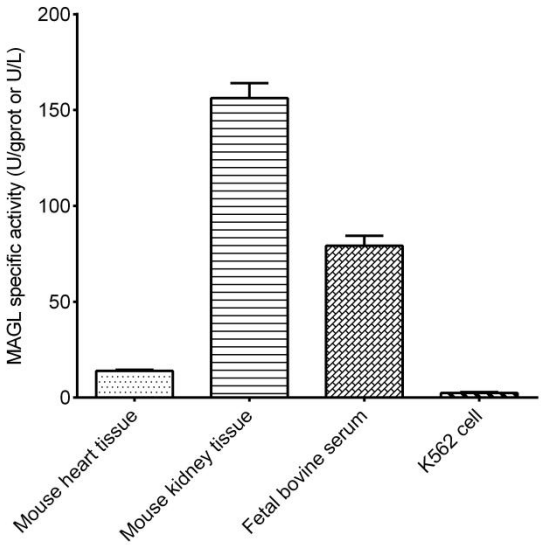
Take 20 μL of 10% mouse heart tissue homogenate which dilute for 5 times and carry the assay according to the operation steps. The results are as follows:

Standard curve: y = 1.3623 x + 0.0062, the average OD value of the sample is 0.714, the average OD value of the control is 0.479, the concentration of protein is 10.14 gprot/L , and the calculation result is:

MAGL activity (U/gprot) = (0.714 - 0.479 - 0.0062) ÷ 1.3623 ÷ 5 × 5 ÷ 10.14 × 1000

=16.56 U/gprot

Detect 10% mouse heart tissue homogenate (the concentration of protein is 10.14 gprot/L, dilute for 5 times), 10% mouse brain tissue homogenate (the concentration of protein is 12.53 gprot/L, dilute for 200 times), fetal bovine serum (dilute for 20 times), 1×10^6 K562 cells (the concentration of protein is 5.05 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.