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

Catalog No：E-BC-K795-M

Specification：48T (46 samples)/96T (94 samples)

Measuring instrument：Microplate reader (330-350 nm)

Detection range：0.67-24.65 U/L

Elabscience® 3-Hydroxy-3-Methylglutaryl Coenzyme A Reductase (HMGR) 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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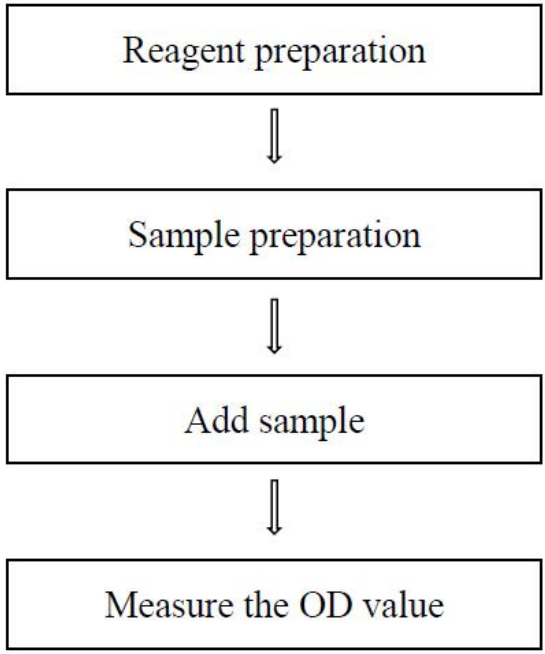
Assay summary



Intended use

This kit can measure 3-hydroxy-3-methylglutaryl coenzyme A reductase activity in animal tissue samples.

Detection principle

3-Hydroxy-3-methyl glutaric acyl coenzyme A reductase (hydroxy methyl glutaric acyl coenzyme A reductase, HMGR) is a hydroxy valeric acid way to control of the rate of enzyme, A hydroxy valeric acid way is acetyl CoA - produces cholesterol metabolic pathways, HMGR detection kit is cholesterol and other related metabolic pathways important means of basic research.

The detection principle of this kit is as follows: HMGR catalyzes the substrate reaction to consume NADPH, causing a decrease in absorbance at 340 nm, and the activity of HMGR can be calculated by measuring its decline rate.

Kit components & storage

Item	Component	Size 1(48 T)	Size 2(96 T)	Storage
Reagent 1	Extraction Solution A	60 mL × 1 vial	60 mL × 2 vials	-20°C, 12 months, shading light
Reagent 2	Extraction Solution B	0.6 mL × 1 vial	1.2 mL × 1 vial	-20°C, 12 months, shading light
Reagent 3	Buffer Solution	10 mL × 1 vial	20 mL × 1 vial	-20°C, 12 months, shading light
Reagent 4	Accelerant	Powder×2 vials	Powder×4 vials	-20°C, 12 months, shading light
Reagent 5	Substrate	0.12 mL×1 vial	0.24 mL×1 vial	-20°C, 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 (330-350 nm, optimum wavelength: 340 nm)

Reagent preparation

① Equilibrate all the reagents to 25℃ before use.

② The preparation of extraction working solution:

Before testing, please prepare sufficient extraction working solution according to the test wells. For example, prepare 500 μL of extraction working solution (mix well 495 μL of extraction solution A and 5 μL of extraction solution B). Keep it on ice protected from light and used up within 1 day.

③ The preparation of accelerant working solution:

Dissolve one vial of accelerant with 0.72 mL of double distilled water, mix well to dissolve. Store at -20°C for 3 days protected from light.

④ The 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 10 μL of substrate and 90 μL of buffer solution). Keep it on ice protected from light and used up within 1 day.

⑤ The preparation of reaction working solution:

Before testing, please prepare sufficient reaction working solution according to the test wells. For example, prepare 450 μL of reaction working solution (mix well 350 μL of buffer solution, 50 μL of accelerant working solution, 50 μL of

substrate working solution). Before use, place the prepared working solution at 25℃at least 5 min. Keep it on ice protected from light and used up within 1 day.

Sample preparation

① Sample preparation

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 working solution 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. Detected the prepared sample within 4h.

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 homogenate	1
10% Mouse brain tissue homogenate	1
10% Mouse lung tissue homogenate	1
10% Mouse kidney tissue homogenate	1
10% Mouse heart tissue homogenate	1
10% Mouse calf muscle tissue homogenate	1

Note: The diluent is extraction working 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 buffer solution to each well.
Sample well: Add 20 μL of samples to each well.

② Add 180 μL of reaction working solution to each well.

③ Mix fully with microplate reader for 5 s and measure the OD value of each well at 340 nm with microplate reader,as A_1.

④ Incubate at 25°C for 20 min, measure the OD value of each well at 340 nm with microplate reader, as A_2.

Calculation

The sample:

For tissue samples:

Definition: The amount of enzyme in 1 kg tissue per 1 min that consume 1 μmol NADPH at 25°C is defined as 1 unit.

$$\text{HMGR activity (U/kg wet weight)} = \frac{\Delta A_{340}}{t \times 0.6 \times \varepsilon} \times \frac{V_{total}}{V_{sample}} \div m \times V \times f$$

[Note]

ΔA_{340}: Absolute OD value of the sample($A = A_1 - A_2$，$\Delta A_{340} = \Delta A_{sample} - \Delta A_{blank}$).

t: Reaction time, 20min.

0.6: Optical path of microplate wells, cm.

ε: The molar extinction coefficient of at 340 nm,16.22×10^-3 L/(μmol•cm).

V_{total}： The volume of reaction system, 0.2 mL.

V_{sample}： The volume of sample, 0.02 mL.

m: The weight of tissue, kg.

V: The volume of extraction solution, L.

f: Dilution factor of sample before test.

Appendix I Performance Characteristics

1. Parameter:

Intra-assay Precision

Three rat heart tissue 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)	5.00	10.00	15.00
%CV	4.2	5.2	6.0

Inter-assay Precision

Three rat heart tissue 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)	5.00	10.00	15.00
%CV	4.2	10.0	8.4

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%.

	Sample 1	Sample 2	Sample 3
Expected Conc. (U/L)	5	10	15
Observed Conc. (U/L)	4.8	9.7	15.0
Recovery rate (%)	95	97	100

Sensitivity

The analytical sensitivity of the assay is 0.67 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 II Example Analysis

Example analysis：

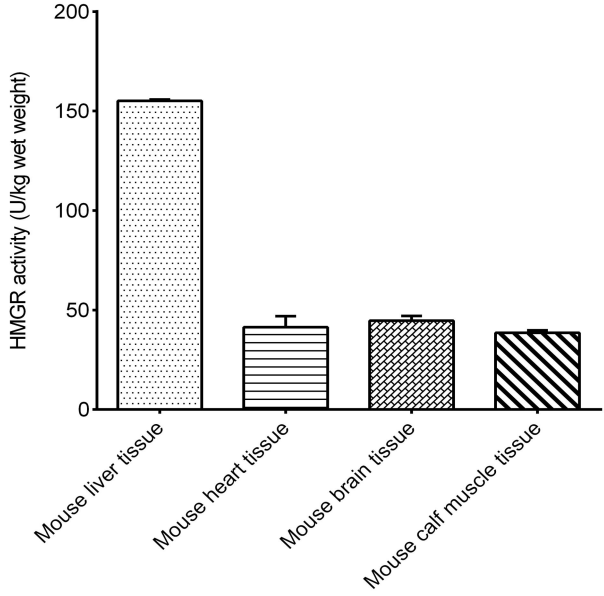
Take 20 μL of 10% mouse liver tissue homogenate, and carry the assay according to the operation steps. The results are as follows:

The A_1 value of the sample is 1.451, the A_2 value of the sample is 1.292, ΔA_{sample} = 1.451 - 1.292 = 0.159; the A_1 value of the blank is 0.825, the A_2 value of the blank is 0.803, ΔA_{blank} = 0.825 - 0.803 = 0.022, $\Delta A_{340} = \Delta A_{sample} - \Delta A_{blank}$ = 0.159 - 0.022 = 0.137, and the calculation result is:

$$\text{HMGR activity (U/kg wet weight)} = 0.137 \div 20 \div 0.6 \div 6.22 \times 1000 \times 0.2 \div 0.02 \div 0.1 \times 1000 \times 0.9 \times 0.001$$

$$= 165.19 \text{ U/kg wet weight}$$

Detect 10% mouse liver tissue homogenate, 10% mouse heart tissue homogenate, 10% mouse brain tissue homogenate, 10% mouse calf muscle tissue homogenate, 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.