CheKine™ Micro Hydroxymethylglutaryl Coenzyme A Synthase (HMGCS) Activity Assay Kit

Cat #: KTB3028 Size: 48 T/48 S 96 T/96 S

	Micro Hydroxymethylglutaryl Coenzyme A Synthase (HMGCS) Activity Assay Kit		
REF	**Cat #:** KTB3028	LOT	**Lot #:** Refer to product label
	Applicable samples: Animal and Plant Tissues, Cells or Bacteria, Fungus, Plasma, Serum or other Liquid samples		
	Storage: Stored at -20℃ for 6 months, protected from light		

Assay Principle

The mevalonate pathway is a very important metabolic pathway, which is involved in the synthesis of many important terpenes precursors and is an important pathway for terpenes biosynthesis. Hydroxymethylglutaryl CoA synthetase (HMGCS) catalyzes acetylCoA and acetoacetylCoA to produce hydroxymethylglutaryl CoA, which is a key step in the biosynthesis of cholesterol and isoprene-like. CheKine™ Micro Hydroxymethylglutaryl Coenzyme A Synthase (HMGCS) Activity Assay Kit can be used to detect biological samples such as animal and plant tissues, cells or bacteria, fungus, plasma, serum or other liquid samples. In the kit, HMGCS catalyzes the formation of hydroxymethylglutaryl CoA with acetoacetyl CoA, and simultaneously produces CoASH, which converts DTNB into yellow TNB, with characteristic absorbance values at 412 nm.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	48 T	96 T	
Extraction Buffer	60 mL	60 mL×2	4℃
Reagent Ⅰ	Powder×1 vial	Powder×1 vial	-20℃, protected from light
Reagent Ⅱ	Powder×1 vial	Powder×1 vial	-20℃, protected from light
Reagent Ⅲ	3 mL	6 mL	4℃, protected from light

Note: Before formal testing, it is recommended to select 2-3 samples with large expected differences for pre-experiment.

Materials Required but Not Supplied

- Microplate reader or visible spectrophotometer capable of measuring absorbance at 412 nm
- 96-well plate or microglass cuvette, precision pipettes, disposable pipette tips, 1.5 mL EP tube
- Incubator, ice maker, freezing centrifuge
- Deionized water
- Homogenizer or mortar (for tissue samples)

Reagent Preparation

Extraction Buffer: Ready to use as supplied; Equilibrate to room temperature before use; Store at 4℃.

Working Reagent Ⅰ: Prepared before use. 48 T add 1.5 mL deionized water, 96 T add 3 mL deionized water to fully dissolve; The remaining reagent can also be stored at -20℃ and protected from light for 1 month after aliquoting to avoid repeated freezing and thawing.

Working Reagent Ⅱ: Prepared before use. 48 T add 1.5 mL deionized water, 96 T add 3 mL deionized water to fully dissolve; The remaining reagent can also be stored at -20℃ and protected from light for 1 month after aliquoting to avoid repeated freezing and thawing.

Reagent Ⅲ: Ready to use as supplied; Equilibrate to room temperature before use; Store at 4℃, protected from light.

Sample Preparation

Note: We recommend that you use fresh samples. If not assayed immediately, samples can be stored at -80℃ for one month.When measuring, the temperature and time of thawing should be controlled. When thawing at room temperature, the sample should be thawed within 4 h.

1. Tissues: Weigh 0.1 g tissue, add 1 mL Extraction Buffer and homogenize or mortar on ice. Centrifuge at 8,000 g for 10 min at 4℃. Use supernatant for assay.
2. Cell, Bacteria or Fungus: Collect 5×10^6 cell, bacteria or fungus into the centrifuge tube, wash cell, bacteria or fungus with cold PBS, discard the supernatant after centrifugation; add 1 mL Extraction Buffer to ultrasonically disrupt the cell, bacteria or fungus 5 min (power 20% or 200 W, ultrasonic 3 s, interval 7 s, repeat 30 times). Centrifuge at 8,000 g for 10 min at 4℃. Use supernatant for assay.
3. Plasma, Serum or other Liquid samples: Test directly.

Note: If the protein concentration of the sample is need to determined, it is recommended to use Abbkine Cat #: KTD3001 Protein Quantification Kit (BCA Assay) to measure the protein concentration of the sample.

Assay Procedure

1. Preheat the microplate reader or visible spectrophotometer for more than 30 min, and adjust the wavelength to 412 nm. Visible spectrophotometer was returned to zero with deionized water.
2. Sample measurement. (The following operations are operated in 96-well plate or microglass cuvette **in turn**)

Reagent	Blank Well (μL)	Test Well (μL)
Working Reagent Ⅰ	25	25
Working Reagent Ⅱ	25	25
Reagent Ⅲ	50	50
Extraction Buffer	100	0
Sample	0	100

3. Mix quickly after adding sample , detect the absorbance A_1 at 10 s immediately at 412 nm and the absorbance A_2 after incubate 4 min 10 s at 25℃ at 412 nm. The Blank Well is recorded as A_{Blank}, the Test Well is marked as A_{Test}. Finally calculate $\Delta A_{Test}=A_{2Test}-A_{1Test}$, $\Delta A_{Blank}=A_{2Blank}-A_{1Blank}$, $\Delta A=\Delta A_{Test}-\Delta A_{Blank}$.

Note: The Blank Well only need to be done 1-2 times. In order to guarantee the accuracy of experimental results, need to do a pre-experiment with 2-3 samples. If ΔA is less than 0.01, increase the sample quantity or extend reaction time appropriately (No more than 8 min). If ΔA is greater than 0.6, the sample can be appropriately diluted with Extraction Buffer, the calculated result multiplied by the dilution factor, or decrease the sample quantity appropriately.

Data Analysis

Note: We provide you with calculation formulae, including the derivation process and final formula. The two are exactly equal. It is suggested that the concise calculation formula in bold is final formula.

Calculation of HMGCS activity:

A. 96-well UV plates calculation formula as below

(1) Calculated by protein concentration

Active unit definition: The production of 1 nmol of TNB per milligram of protein per min was defined as one unit of enzyme activity.

HMGCS (U/mg prot)=[ΔA×V_{Total}÷(ε×d)×10^9]÷(V_{Sample}×Cpr)÷T**=73.53×ΔA÷Cpr**

(2) Calculated by fresh weight of samples

Active unit definition: The production of 1 nmol of TNB of NADH per gram tissue per min was defined as one unit of enzyme activity.

HMGCS (U/g fresh weight)=[ΔA×V_{Total}÷(ε×d)×10^9]÷(W×V_{Sample}÷$V_{Total\ sample}$)÷T**=73.53×ΔA÷W**

(3) Calculated by cells, bacteria or fungus

Active unit definition: The production of 1 nmol of TNB per 10^4 cells, bacteria or fungus per min was defined as one unit of enzyme activity.

HMGCS (U/10^4)=[ΔA×V_{Total}÷(ε×d)×10^9]÷(n×V_{Sample}÷$V_{Total\ sample}$)÷T**=73.53×ΔA÷n**

(4) Calculated by volume of liquid samples

Active unit definition: The production of 1 nmol of TNB per mL liquid was defined as one unit of enzyme activity.

HMGCS (U/mL)=[ΔA×V_{Total}÷(ε×d)×10^9]÷(V_{Sample}÷$V_{Total\ sample}$)÷T**=73.53×ΔA**

V_{Total}: total reaction volume, 0.2 mL=0.0002 L; ε: TNB molar extinction coefficient, 1.36×10^4 L/mol /cm; d: the light path of the 96-well plate, 0.5 cm; 10^9: 1 mol=1×10^9 nmol; V_{Sample}: sample volume added, 0.1 mL; $V_{Total\ sample}$: added Extraction Buffer volume , 1 mL; T: reaction time, 4 min; Cpr: sample protein concentration, mg/mL; W: weight of sample;g. n: Number of cells, bacteria or fungus, calculated in units of million.

B. Microquartz cuvette calculation formula

The optical diameter d: 0.5 cm in the above calculation formula can be adjusted to d: 1 cm for calculation.

Typical Data

The following data are for reference only. And the experimenters need to test the samples according to their own experiments.

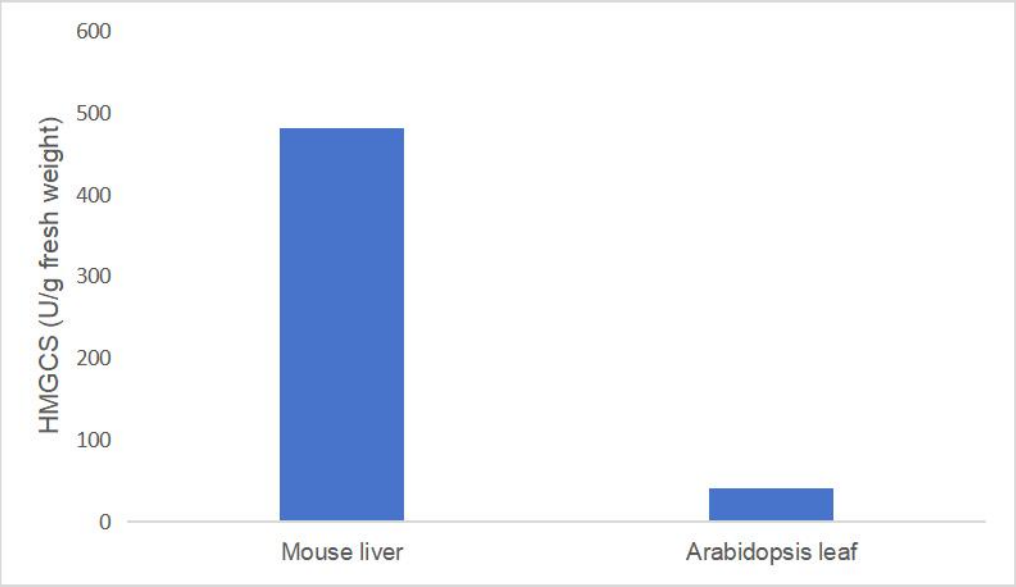

Figure 1. Determination of HMGCS activity in mouse liver and arabidopsis leaf by this kit.

Recommended Products

Catalog No.	Product Name
KTB1410	CheKine™ Micro Alanine Aminotransferase (ALT/GPT) Activity Assay Kit
KTB1420	CheKine™ Micro Aspartate Aminotransferase (AST/GOT) Activity Assay Kit
KTB1430	CheKine™ Micro Proline (PRO) Content Assay Kit

Disclaimer

The reagent is only used in the field of scientific research, not suitable for clinical diagnosis or other purposes. For your safety and health, please wear a lab coat and disposable gloves.