Hydroxymethylglutaryl coenzyme A synthase Activity Assay Kit

Note: Take two or three different samples for prediction before test.

Operation Equipment: Spectrophotometer/ Microplate Reader

Catalog Number: BC5435

Size: 100T/96S

Components:

Extract solution: Liquid 110ml×1, store at 2-8℃.

Reagent I: Powder×1, store at -20℃. Dissolve well with 1mL of distilled water before use. Unused reagents can be stored at -20°C for up to 4 weeks, avoid repeated freezing and thawing.

Reagent I working solution**:** According to sample number compound according to the ratio of reagent I: distilled water = 20 μL: 460 μL (480 μL,about 9 T). Prepare the working solution before use and use it up the same day.

Reagent II: Powder×1, store at -20℃. Dissolve well with 1mL of distilled water before use. Unused reagents can be stored at -20°C for up to 4 weeks, avoid repeated freezing and thawing.

Reagent II working solution**:** According to sample number compound according to the ratio of reagent I: distilled water = 30 μL: 330 μL (360 μL,about 9 T). Prepare the working solution before use and use it up the same day.

Reagent III: Liquid 6ml×1, store at 2-8℃.

Product Description:

The mevalonate pathway is a very important metabolic pathway involved in the synthesis of precursors of many important terpenoids and is an important pathway in terpenoid biosynthesis. Hydroxymethylglutaryl coenzyme A synthase catalyzes the formation of acetyl coenzyme A and acetoacetyl coenzyme A to produce hydroxymethylglutaryl coenzyme A, a key step in the biosynthesis of cholesterol and isoprenoids.

Hydroxymethylglutaryl coenzyme A synthase catalyzes the formation of hydroxymethylglutaryl coenzyme A from acetyl coenzyme A and acetoacetyl coenzyme A. It also produces CoASH, which converts DTNB to TNB (yellow), with characteristic absorbance values at 412 nm.

Reagents and Equipment Required but Not Provided:

Spectrophotometer/ microplate Reader, low temperature centrifuge, adjustable transferpettor, water bath/ constant temperature incubator, micro glass cuvettes/96 well plates, mortar/homogenizer/cell ultrasonicator , ice and distilled water.

I. Sample extraction:

1. Tissue:

Accordance the ratio of tissue(g) : extract solution volume (mL)=1: 5~10 (add 1 mL of extract solution to 0.1 g of tissue), homogenate on ice. Centrifuge at 8000g for 10 minutes at 4℃, take the

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supernatant and place it on ice for testing.

2. Bacteria or cells:

Accordance the ratio of cells amount(10^6) : extract solution volume (mL)=5~10: 1 (add 1 mL of extract solution to 5 million cells). Ultrasonic on ice bath to smash cells, (powder 200w, ultrosonic 3s, interval 10s for 5 minutes). Centrifuge at 8000g for 10 minutes at 4°C, take the supernatant and place it on ice for testing.

II. Determination procedure:

1. Preheat spectrophotometer/ microplate Reader for 30min, adjust the wavelength to 412 nm, spectrophotometer set the counter to zero with distilled water.
2. depending on the number of samples, take some of the reagent I working solution, the reagent II working solution and reagent III preheated at 37°C for 10min.
3. Add the following reagents: (Add the following reagents to the glass cuvette)

Reagent name（μL）	Test tube (T)	Contrast tube (C)
reagent I working solution	25	25
reagent II working solution	25	25
reagent III	50	50
Sample	100	-
Extraction	-	100
Add the above reagents to a 1 mL glass cuvette and mix thoroughly, measure the absorbance A1 at 412nm for 10s, quickly place in a 37°C water bath or constant temperature incubator for 20min, wipe dry quickly and measure the absorbance A2 at 10s for 20min, record the absorbance A1 at 10s at 412nm and the absorbance A2 after 20min. calculate ΔAt= A2t -A1t, ΔAb = A2b- A1b, ΔA = ΔAt - ΔAb. Blank tubes should only be done 1-2 times.		

III. Calculation of HMGCS activity:

A. Calculated from micro quartz cuvettes:

1 Protein concentration:

Unit definition: One unit of enzyme activity is defined as the amount of enzyme that catalytic production of 1 nmol TNB per min every. mg tissue protein

HMGCS activity（U/mg prot）=ΔA÷（ε×d）×10^9×Vr÷（Vs×Cpr）÷T=7.353×ΔA÷Cpr

2 Sample weight:

Unit definition: One unit of enzyme activity is defined as the amount of enzyme that catalytic production of 1 nmol TNB per min every gram tissue weight.

HMGCS activity（U/g weight）= ΔA×Vr÷（ε×d）×10^9÷（W ×Vs÷Ve）÷T=7.353×ΔA÷W

3 Cells or bacteria:

Unit definition: One unit of enzyme activity is defined as the amount of enzyme that catalytic production of 1 nmol TNB per min every 10^6 cells.

HMGCS activity（U/10^6 cell）= ΔA×Vr÷（ε×d）×10^9÷（N×Vs÷Ve）÷T=7.353×ΔA÷N

B. Calculated from micro quartz cuvettes:

Replace d-1cm in the above equation with d-0.6cm (96-well plate optical diameter) for calculation

Vr： Total volume of reaction system， 1×10^{-3}L； ε： TNB molar extinction coefficient， 1.36×10^{4}L/mol/cm；d：cuvette light diameter，1cm；Vs：Volume of sample added，0.5mL；Ve：Volume of extraction solution added，1mL；T：Reaction time，20min；Cpr：Sample protein concentration，mg/mL；W：Sample quality，g；N：Total number of cells or bacteria，Counting in 10^{6}

Note:

1. If the ΔA absorbance value is too low or close to blank, extend the reaction time or increase the sample size as appropriate and re-determine. Note the simultaneous modification of the calculation formula.
2. If A2 > 1 or ΔA > 0.8, it is recommended to dilute the sample appropriately or shorten the reaction time for determination. Note the simultaneous modification of the calculation formula.

Experimental Examples:

1. Weigh 0.1046 g of pleurotus ostreatus, add the extract for ice bath homogenization, the extract dilute the supernatant 2 times, operate according to the assay procedure, measure with a 1 mL glass cuvette to calculate ΔAt= A2t - A1t ==0.906-0.652=0.254, ΔAb = A2b - A1b =0.121-0.104=0.017, ΔA = ΔAt- ΔAb= 0.237, calculating enzyme activity by sample mass gives

   HMGCS activity (U/g mass) =12.255×ΔA÷W=27.767 U/g mass

2. 0.1070 g of green pepper was weighed, added to the extract for homogenisation in an ice bath and operated according to the assay procedure, measured using a 1 mL glass cuvette to calculate ΔAt= A2t - A1t=0.342-0.240=0.102, ΔAb= A2b- A1b=0.121-0.104=0.017, ΔA = ΔAt - ΔAb=0.085, calculation of enzyme activity by sample mass gives:

   HMGCS activity (U/g mass) =12.255×ΔA÷W=9.735 U/g mass

References：

[1] Skaff D A, Miziorko H M. A visible wavelength spectrophotometric assay suitable for high-throughput screening of 3-hydroxy-3-methylglutaryl-CoA synthase[J]. Analytical Biochemistry, 2010, 396(1):96-102

[2] Scharnagl H, W März, Schliack M, et al. A novel assay for cytosolic 3-hydroxy-3-methylglutaryl-coenzyme A synthase activity using reversed-phase ion-pair chromatography: demonstration that Lifibrol (K12.148) modulates the enzyme activity[J]. Journal of Lipid Research, 1995, 36(3):622-627.

Related Products:

BC0550/BC0555	Fatty Acid Synthase Activity Assay Kit
BC0590/BC0595	Free Fatty Acids (FFA) Content Assay Kit
BC0620/BC0625	Triglyceride(TG) Content Assay Kit
BC1890/BC1895	Free Cholestenone(FC) Content Assay Kit
BC1980/BC1985	Total cholesterol (TC) Content Assay Kit
BC2340/BC2345	Lipase(LPS) Assay Kit

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