Methylcitrate Synthase（MCS）Activity Assay Kit

Detection Equipment: Spectrophotometer

Catalog Number: BC5860

Size: 50T/24S

Components:Please carefully check the volume of the reagent and the volume in the bottle before use. If you have any questions, please contact Solarbio staff in time.

Reagent name	Size	Preservation condition
Extract	Liquid 30 mL×1	-20℃ storage
Reagent I	Liquid 0.3 mL×1	-20℃ storage
Reagent II	Liquid 60 mL×1	2-8℃ storage
Reagent III	Liquid 2.5 mL×1	2-8℃ storage
Reagent IV	Powder ×1	-20℃ storage
Reagent V	Powder ×1	-20℃ storage

Solution reparation :

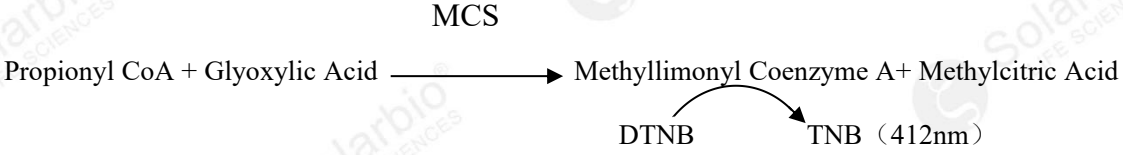
1. Reagent IV: Add 1.5mL distilled water to fully dissolve before use. The unused reagent is storage at -20℃ for 4 weeks to avoid repeated freezing and thawing.

2. Reagent V: Put the reagent in a glass bottle in a brown bottle, add 3mL distilled water to fully dissolve it before use, and storage the unused reagent at -20℃ for 4 weeks to avoid repeated freezing and thawing.

Description:

Methylcitrate Synthase (MCS) is widely present in the mitochondrial matrix of animals, plants, microorganisms and cultured cells, and is involved in the regulation of tricarboxylic acid cycle together with citrate synthase (CS).

MCS catalyzes propionyl CoA and oxaloacetic acid to produce methyllimonyl Coenzyme A, which further hydrolyzes to produce methylcitric acid. The reaction transforms colorless DTNB into yellow TNB with a characteristic absorption peak at 412nm, from which MCS activity can be calculated.



Note: Before the experiment, it is recommended to select 2-3 sample with large expected differences for pre-experiment. If the absorbance value of the sample is not within the measurement range, it is recommended to dilute or increase the sample size for detection.

Reagents and Equipment Required but Not Provided:

Spectrophotometer, 1mL quartz cupola, balance, low temperature centrifuge, water bath, mortar/homogenizer/cell ultrasonic crusher, ice and distilled water.

Protocol:

I. Sample processing (The sample size to be tested can be appropriately adjusted, and the specific proportion can be referred to the literature)

1. Tissue: According to the ratio of tissue mass (g) : extraction liquid volume (mL) of 1:5-10 (it is recommended to weigh about 0.1g of tissue and add 1 ml of extraction liquid) and 10μL of reagent 1 for ice bath homogenization. The sample were centrifuged at 12000g for 10min at 4℃, and the supernatant was removed and placed on ice until measured.

2. Bacteria/cell: According to the number of bacteria/cell (10^6) : the ratio of extraction liquid volume (mL) to 5~ 10:1 (it is recommended that 5 million bacteria/cell add 1mL extraction solution and 10μL reagent 1), the ice bath ultrasonic fragmentation (power 200W, ultrasonic 3 s, interval 7 s, total time 5 min); Then the sample were centrifuged at 12000 g for 10min at 4℃, and the supernatant was removed and placed on ice until measured.

II. Measurement Steps

1. It can be seen that the spectrophotometer is preheated for more than 30min, the wavelength is adjusted to 412nm, and zero it with distilled water.
2. Preheat Reagent II at 37℃ for 15min.
3. Operation table: Add to 1mL glass cuvette or 1.5mL EP tube (Suitable for measuring animal tissue sample).

Reagent name（μL）	Control tube	Test tube
Reagent II	930	800
Reagent III	35	35
Reagent IV	-	40
Sample	35	35
Reagent V	-	90
The reagents mentioned above were added into a 1mL glass cuvette or a 1.5mL EP tube, and the initial absorbance A1 $_{control}$ and A1 $_{text}$ at 412nm for 10s were recorded after thorough mixing. Then the cuvette was quickly placed in a 37℃ water bath with the reaction solution for 5min, and the cuvette was quickly removed and dried. Absorbance A2 $_{control}$ and A2 $_{text}$ at 5min10s were recorded at 412nm, and ΔA= (A2 $_{text}$ -A1 $_{text}$) - (A2 $_{control}$-A1 $_{control}$) was calculated.		

4. Operation table: Add successively in the microcolorimetric dish / 96-well plate(suitable for the determination of microbial and plant tissue sample)

Reagent name（μL）	Control tube	Test tube
Reagent II	765	635
Reagent III	35	35
Reagent IV	-	40
Sample	200	200
Reagent V	-	90
The reagents mentioned above were added into a 1mL glass cuvette or a 1.5mL EP tube, and the initial absorbance A1 $_{control}$ and A1 $_{text}$ at 412nm for 10s were recorded after thorough mixing.		

Then the cuvette was quickly placed in a 37℃ water bath with the reaction solution for 30min, and the cuvette was quickly removed and dried. Absorbance A2 $_{control}$ and A2 $_{text}$ at 30min10s were recorded at 412nm, and ΔA= (A2 $_{text}$ -A1 $_{text}$) - (A2 $_{control}$-A1 $_{control}$) was calculated.

Note: When performing the reaction in a 1.5mL EP tube, refer to the following steps: Then the reaction solution was mixed and quickly added to a 1mL glass cuvette, and the initial absorbance A1 $_{control}$ and A1 $_{text}$ at 412nm for 10s were recorded. Then the reaction solution was quickly sucked into a 1.5mL EP tube, placed in a 37℃ water bath for 30 minutes, and the EP tube was quickly removed and dried. Absorbance A2 $_{control}$, A2 $_{text}$ at 10s for 30min was recorded at 412nm.

III. Calculations

1. Animal tissue sample:

1) Calculated by sample protein concentration:

Enzyme activity definition: The reduction of 1nmol TNB per mg of histone per minute in the reaction system is defined as one unit of enzyme activity.

PPDK activity (U/mg prot) =ΔA÷ (ε×d) ×V $_{reaction}$÷(Cpr÷V $_{sample}$)÷T =420.17×ΔA÷Cpr

2) Calculated by sample quality:

Enzyme activity definition: The reduction of 1nmol TNB per minute per g of tissue in the reaction system is defined as one unit of enzyme activity.

PPDK activity (U/g) =ΔA÷(ε×d)×V $_{reaction}$÷(W÷V $_{extractionion}$×V $_{sample}$)÷T = 424.37×ΔA÷W

ε: TNB molar extinction coefficient,13.6×10^{-3} mL/(nmol·cm);

d: light diameter of cuvette, 1cm;

V $_{reaction}$: reaction system volume, 1mL;

V $_{sample}$: the sample volume added to the reaction system, 0.035mL;

V extraction$_{ion}$: add the volume of extract and Reagent I, 1.01mL;

T: reaction time, 5min;

Cpr: sample protein concentration, mg/mL;

W: sample quality, g.

2. Microbial and plant tissue sample:

1) According to the sample protein concentration:

Enzyme activity Definition: One unit of enzyme activity was defined as 1nmol TNB per minute catalyzed by 1 mg of tissue protein in the reaction system.

MCS activity (U/mg prot) =ΔA÷ (ε×d) ×V $_{reaction}$ ÷ (Cpr× V $_{sample}$) ÷T=12.25×ΔA÷Cpr

2) According to sample quality:

Enzyme activity Definition: Production of 1nmol TNB per minute per g of tissue in the reaction system was defined as one unit of enzyme activity.

MCS activity (U/g mass) =ΔA÷ (ε×d) ×V $_{reaction}$ ÷ (W÷V $_{extraction}$ × V $_{sample}$) ÷T=12.38×ΔA÷W

3) According to bacteria/cell:

Enzyme activity definition: 1nmol TNB per minute catalyzed by 10^6 bacteria/cell in the reaction system was defined as one unit of enzyme activity.

MCS activity (U/10^6 cells) =ΔA÷ (ε×d) ×V $_{reaction}$ ÷ (N÷V $_{extraction}$ × V $_{sample}$) ÷T = 12.38×ΔA÷N

ε : TNB molar extinction coefficient, 13.6×10^{-3}mL/(nmol·cm);
d: light diameter of cuvette, 1cm;
$V_{reaction}$: reaction system volume, 1mL;
V_{sample}: the sample volume added to the reaction system, 0.2mL;
$V_{extractionion}$: add the volume of extract and reagent 1, 1.01mL;
T: reaction time, 30min;
Cpr: sample protein concentration, mg/mL;
W: sample quality, g;
N: Total number of bacteria or cells, calculated as 10^6.

Note:

1. The sample and all reagents are placed on ice during the determination process to avoid denaturation and inactivation.
2. It is best for two people to do this experiment at the same time, one person to compare colors and one person to time, in order to ensure the accuracy of the experimental results.
3. Because the extract contains protein components (about 1mg/mL), it is necessary to measure the protein concentration of the extract at the same time.
4. When the sample ΔA < 0.01, the enzymatic reaction time can be appropriately extended or the sample size can be increased before the determination, and the calculation formula can be modified simultaneously.
5. When the sample ΔA>1 or A>1.5, the sample can be properly diluted with the extraction solution and measured, and pay attention to the simultaneous modification of the calculation formula.

Experimental example:

1. Take 0.0918g mouse heart and add 1mL extract solution and 10μL reagent to the ice bath homogenate. Take supernatant and use it according to the determination procedure, and use 1 mL glass cuvette to measure ΔA= ($A2_{text}$ -$A1_{text}$) - ($A2_{control}$ -$A1_{control}$) = (0.930-0.339) - (0.333-0.294) =0.552. MCS activity calculated by sample mass was as follows:
   MCS activity (U/g mass) = 424.37×ΔA÷W =2551.76U/g mass.
2. Take 0.1186g mold and add 1mL extract solution and 10μL Reagent I for ice bath homogenization. After taking superqing, follow the determination procedure, and use 1 mL glass cuvette to measure ΔA= ($A2_{text}$ -$A1_{text}$) - ($A2_{control}$ -$A1_{control}$) = (0.536-0.396) - (0.357-0.376) =0.159. The MCS activity calculated by sample mass is as follows:
   MCS activity (U/g mass) =12.38×ΔA÷W =16.60 U/g mass.
3. Take 0.1013g bamboo leaves and add 1mL extraction solution and 10μL Reagent I for ice bath homogenization. After taking superqing, follow the determination steps and use 1 mL glass cuvette to measure ΔA= ($A2_{text}$ -$A1_{text}$) - ($A2_{control}$ -$A1_{control}$) = (0.477-0.413) - (0.411-0.403) =0.056. The MCS activity calculated by sample mass is as follows:
   MCS activity (U/g mass) =12.38×ΔA÷W =6.844 U/g mass.

References：

[1] Maerker, C, et al. "Methylcitrate synthase from Aspergillus fumigatus. Propionyl-CoA affects polyketide synthesis, growth and morphology of conidia. " Febs Journal 272.14(2010):3615-3630.

[2] Watson, D., Lindel, D.L. & Fall, R. Pseudomonas aeruginosa contains an inducible methylcitrate synthase. Current Microbiology 8, 17–21 (1983).

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