Malic Acid Synthase (MS) Activity Assay Kit

Detection Equipment: Spectrophotometer/Microplate Reader

Catalog Number: BC5765

Size: 100T/48S

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 60 mL×1	2-8℃ storage
Reagent I	Liquid 15 mL×1	2-8℃ storage
Reagent II	Liquid 600 μL×1	2-8℃ storage
Reagent III	Powder ×1	-20℃ storage
Reagent IV	Liquid 1.1 mL×1	2-8℃ storage
Reagent V	Liquid 6 mL×1	2-8℃ storage
Reagent VI	Liquid 1.3 mL×1	2-8℃ storage

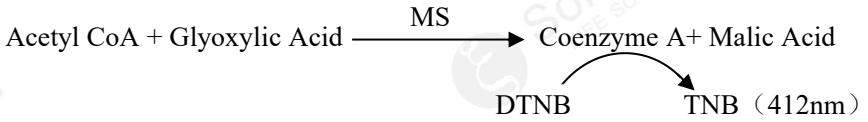
Solution reparation :

1. Reagent III: Before use, add 1.5mL distilled water to fully dissolve, and store the unused reagent for 4 weeks at -20℃ to avoid repeated freezing and thawing.

Description:

Malate Synthase (EC2.3.3.9) belongs to a class of acyltransferases in transferases and mainly exists in plants and microorganisms. It is one of the key enzymes in the glyoxylate cycle. Glyoxylate reacts with acetyl-coa to form malate under the catalysis of MS.

MS catalyzes the production of malic acid from acetyl CoA and glyoxylate, along with the production of coenzyme A, which converts colorless DTNB to yellow TNB. At 412nm, there are characteristic absorption peaks at, from which MS 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/microplate reader, micro glass cuvette/96 well flat-bottom plate, balance, cryogenic centrifuge, water bath, mortar/homogenizer/cell ultrasonicator, 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) for ice bath

homogenization.

The semple were centrifuged **2. Bacteria/cell:** According to the nat 12000g for 10 min at 4℃, and the supernatant was removed and placed on ice until measured.

umber of bacteria/cell (10^6) : the ratio of the volume of the extraction liquid (mL) is 5~ 10:1 (it is

recommended that 5 million bacteria/cell add 1mL of the extraction solution), the ice bath is broken by ultrasonic (the power is 200W, the ultrasonic is 3 s, the interval is 7 s, the total time is 5 min); Then the semple were centrifuged at 12000 g for 10 min at 4℃, and the supernatant was removed and placed on ice until measured.

3. Culture supernatant and other liquids: Direct detection, if there is turbidity, the supernatant can be taken after centrifugation for determination.

II. Measurement Steps

1. Preheat the visible spectrophotometer for more than 30min, adjust the wavelength to 412nm, and adjust

the distilled water to zero.

2. Preheat Reagent I at 25℃ for 15min.

3. Operation table: (Add the following reagents to 1.5mL EP tube)

1) Enzymatic Reaction

Reagent name（μL）	Control tube	Test tube
Reagent I	140	120
Reagent II	-	10
Reagent III	-	10
Sample	50	50
Thoroughly mixed, react at 25℃ for 20min		
Reagent IV	10	10
Thoroughly mixed, centrifuged at 4℃ 12000g for 5min, supernatant was taken into 1.5mL EP tube.		

2) Color Reaction

Reagent name（μL）	Control tube	Test tube
Supernate	140	140
Reagent V	50	50
Reagent VI	10	10
Thoroughly mixed and allowed to stand for 5min, and measured absorbance at 412nm. Recorded as $A_{control}$ and $A_{determination}$, respectively. $\Delta A=A_{determination}-A_{control}$ was calculated. One monitor is required for each measuring tube.		

III. Calculations

1. Use microcuvette to determine:

1) Calculated by sample protein concentration:

Enzyme activity definition:The catalytic production of 1nmol TNB per min per mg of tissue

protein in the reaction system at 25℃ was defined as one unit of enzyme activity.

MS activity (U/mg prot) =ΔA÷ (ε×d) ×$V_{color\ development}$ ÷$V_{supernatant}$ ×$V_{enzymatic}$ ÷ (Cpr×V_{sample}) ÷T×F =21×ΔA÷Cpr×F

2) Calculated by sample quality:

Enzyme activity definition: The catalytic production of 1nmol TNB per min per g of tissue in the reaction system at 25℃ was defined as one unit of enzyme activity.

MS activity (U/g) = ΔA÷ (ε×d) ×$V_{color\ development}$ ÷$V_{supernatant}$ ×$V_{enzymatic}$ ÷ (W ÷$V_{extraction}$ ×V_{sample})

÷T×F = 21×ΔA÷W×F

3) Calculated by bacteria/cell:

Enzyme activity definition: TThe catalytic production of 1nmol TNB per min per 106 bacteria/cell in

the reaction system at 25℃ was defined as one unit of enzyme activity.

MS activity (U/106 cell) =ΔA÷ (ε×d) ×$V_{color\ development}$ ÷$V_{supernatant}$ ×$V_{enzymatic}$ ÷ (N÷$V_{extraction}$ ×V_{sample})

÷T×F =21×ΔA÷N×F

4) Calculated by liquid volume:

Enzymatic activity definition: The catalytic production of 1nmol TNB per min per mL of liquid in the

reaction system at 25℃ was defined as one unit of enzyme activity.

MS activity (U/mL) = ΔA÷ (ε×d) ×$V_{color\ development}$ ÷$V_{supernatant}$ ×$V_{enzymatic}$ ÷V_{sample} ÷ T× F = 21×ΔA÷N×F

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

d: Light diameter of the cupola, 1cm;

$V_{color\ development}$: Total volume of color reaction, 0.2mL;

$V_{supernatant}$: Supernatant liquid volume in color reaction, 0.14mL;

$V_{enzymatic}$: Total volume of enzymatic reaction, 0.2mL;

V_{sample}: The sample volume added to the reaction system, 0.05mL;

$V_{extraction}$: Add the volume of extraction liquid, 1mL;

T: Reaction time, 20min;

Cpr: Sample protein concentration, mg/mL;

W: Sample quality, g;

N: Total number of bacteria or cells, as 10^6.

2. Use 96-well plates to determine:

1) Calculated by sample protein concentration:

Enzyme activity definition:The catalytic production of 1nmol TNB per min per mg of tissue protein in the reaction system at 25℃ was defined as one unit of enzyme activity.

MS activity (U/mg prot) =ΔA÷ (ε×d) ×$V_{color\ development}$ ÷$V_{supernatant}$ ×$V_{enzymatic}$ ÷ (Cpr×V_{sample}) ÷T×F =35×ΔA÷Cpr×F

2) Calculated by sample quality:

Enzyme activity definition: The catalytic production of 1nmol TNB per min per g of tissue in

the reaction system at 25℃ was defined as one unit of enzyme activity.

MS activity (U/g) = ΔA÷ (ε×d) ×$V_{color\ development}$ ÷$V_{supernatant}$ ×$V_{enzymatic}$ ÷ (W ÷$V_{extraction}$ ×V_{sample})

÷T×F = 35×ΔA÷W×F

3) Calculated by bacteria/cell:

Enzyme activity definition: TThe catalytic production of 1nmol TNB per min per 106 bacteria/cell in

the reaction system at 25℃ was defined as one unit of enzyme activity.

MS activity (U/106 cell) =ΔA÷ (ε×d) ×$V_{color\ development}$ ÷$V_{supernatant}$ ×$V_{enzymatic}$ ÷ (N÷$V_{extraction}$ ×V_{sample})

÷T×F =35×ΔA÷N×F

4) Calculated by liquid volume:

Enzymatic activity definition: The catalytic production of 1nmol TNB per min per mL of liquid in the

reaction system at 25℃ was defined as one unit of enzyme activity.

MS activity (U/mL) = ΔA÷ (ε×d) ×$V_{color\ development}$ ÷$V_{supernatant}$ ×$V_{enzymatic}$ ÷V_{sample} ÷ T× F = 35×ΔA÷N×F

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

d: Light diameter of 96-well plate, 0.6cm;

$V_{color\ development}$: Total volume of color reaction, 0.2mL;

$V_{supernatant}$: Supernatant liquid volume in color reaction, 0.14mL;

$V_{enzymatic}$: Total volume of enzymatic reaction, 0.2mL;

V_{sample}: The sample volume added to the reaction system, 0.05mL;

$V_{extraction}$: Add the volume of extraction liquid, 1mL;

T: Reaction time, 20min;

Cpr: Sample protein concentration, mg/mL;

W: Sample quality, g;

N: Total number of bacteria or cells, as 10^6.

Note:

1. The semple and all reagents were placed on ice during the assay 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, so as to ensure the accuracy of the experimental results.
3. If sample ΔA< 0.01, the sample volume can be appropriately increased to re-extract or increase the sample volume of the sample table (can simultaneously reduce the volume of the reagent to ensure that the total volume is unchanged) before determination; If sample ΔA> 1.0 or A determination > 1.5, the supernatant can be diluted with distilled water for determination, and the dilution multiple in the calculation formula should be modified simultaneously.

Experimental example:

1. Take 0.1141g of mold was added to 1 ml of extract for homogenization in an ice bath, and the

supernatant was removed and followed the determination steps. The $\Delta A=A_{text}-A_{control}$ =0.247-0.206=0.041 was measured by 96-well plate, and the MS activity was calculated according to the sample mass.

MS activity (U/g mass) =35×ΔA÷W×F =12.577 U/g mass.

2. Take 0.1169g of germinated mung beans and add 1mL of extract to the ice bath homogenate, take the supernatant and dilute it with distilled water for 2 times and follow the determination steps. The $\Delta A=A_{text}-A_{control}$ =0.406-0.244=0.162 measured by 96-well plate, and the MS activity is calculated according to the sample mass:

   MS activity (U/g mass) =35×ΔA÷W×F =97.006 U/g mass.

3. Take 0.1102g mango was added to 1mL extract for ice bath homogenization, and the supernatant was taken and followed the determination steps. The $\Delta A=A_{text}-A_{control}$ =0.341-0.153=0.188 was measured by 96-well plate, and the MS activity was calculated according to the sample mass.

   MS activity (U/g mass) =35×ΔA÷W×F =59.710 U/g mass.

References：

[1] Roucourt B, Minnebo N, Augustijns P, Hertveldt K, Volckaert G, Lavigne R. Biochemical characterization of malate synthase G of P. aeruginosa. BMC Biochem. 2009 Jun 24;10:20.

[2] Miernyk JA, Trelease RN, Choinski JS. Malate synthase activity in cotton and other ungerminated oilseeds: a survey. Plant Physiol. 1979 Jun;63(6):1068-71.

Related products:

BC1040	NAD Kinase(NADK) Activity Assay Kit
BC1045	NAD-Malate Dehydrogenase(NAD-MDH) Activity Assay Kit
BC1120	NADP Phosphatase(NADPase) Activity Assay Kit
BC1125	NADP Malic Enzyme(NADP-ME) Activity Assay Kit
BC5490	Fluoride Resistant Acid Phosphatase（FRAP）Activity Assay Kit
BC5495	Fluoride Resistant Acid Phosphatase（FRAP）Activity Assay Kit