

# Malic Acid Content Assay Kit

**Note:** It is necessary to predict 2-3 large difference samples before the formal determination.

**Operation Equipment:** Spectrophotometer

**Catalog Number:** BC5490

**Size:** 50T/24S

## Components:

**Extraction reagent I:** Liquid 30 mL×1. Store at 2-8°C.

**Extraction reagent II:** Liquid 5 mL×1. Store at 2-8°C

**Reagent I:** Liquid 20 mL×1. Store at 2-8°C.

**Reagent II:** Powder ×1. Store at -20°C, Dissolve with 13mL of distilled water before use. It can be stored at 2-8°C for 4 weeks.

**Reagent III:** Liquid 12 mL×2. Store at 2-8°C.

**Reagent IV:** Liquid 50 μL×1. Store at 2-8°C. Before use according to Reagent IV: Reagent IV diluent =10μL: 1mL (about 8T) proportion dilution Reagent IV standby.

**Reagent IV diluent:** Liquid 8 mL×1. Store at 2-8°C.

**Standard goods:** Liquid 1 mL×1. Store at 2-8°C. 100μmol/mL malic acid Standard solution. Before use, 10μL 100μmol/mL malic acid standard solution was taken, and 240μL distilled water was added to mix thoroughly to prepare 4μmol/mL malic acid standard solution. Then take 100μL 4μmol/mL malic acid standard solution, add 900μL distilled water, mix thoroughly to prepare 0.4μmol/mL malic acid standard solution for use. (In the experiment, each tube needs 20μL, so the large volume is prepared to reduce the experimental error).

## Product Description

L-malic acid is an intermediate product of the tricarboxylic acid cycle and an important part of the malate-aspartic acid shuttle. Malate-aspartic acid shuttle is required for the reductive equivalent transmembrane transport process of oxidative phosphorylation. In lower organisms, malic acid is converted to lactic acid in the process of malolactic fermentation, which also produces CO<sub>2</sub>. Malic acid is commonly used as an additive in the food and pharmaceutical industries, and quantitative analysis of malic acid is also vital in the production of beer, wine, cheese and fruit.

Malate dehydrogenase catalyzes malic acid and NAD to form oxaloacetic acid, NADH and NH<sub>4</sub><sup>+</sup>. Under 1-mPMS, WST-1 reacts with NADH to produce water-soluble Formazan. The maximum absorption peak at 450nm can be used to calculate malic acid content.

## Reagents and Equipment Required but Not Provided.

Spectrophotometer, mortar/homogenizer/cell ultrasonic crusher, centrifuge, constant temperature foster box/water-bath, 1 mL glass cuvette, ice and distilled water.

## Procedure:

### I. Sample preparation

1. **Tissue:** According to the proportion of tissue weight (g): Extraction reagent I volume (mL) of 1:5-10 to extract. It is suggested that 0.1 g of tissue with 1 mL of Extraction reagent I and fully homogenized on ice bath. Centrifuge at 12000 g for 10 minutes at 4°C. Take 0.8mL of supernatant, add 0.15mL of Extraction reagent II fully shake and mix, centrifuge at 4°C at 12000g for 10 minutes, take the supernatant on ice before testing.
2. **Cells:** Collect cells into the centrifuge tube, discard supernatant after centrifugation. According to the proportion of cells number ( $10^4$ ): Extraction reagent I volume (mL) of 500-1000:1 to extract. It is suggested that 5 million of cell amount with 1 mL of Extraction reagent I. Split the cell with ultrasonication (placed on ice, ultrasonic power 300W, working time 3s, interval 7s, total times:3 minutes). Centrifuge at 12000 g for 10 minutes at 4°C. Take 0.8mL of supernatant, add 0.15mL of Extraction reagent II fully shake and mix, centrifuge at 4°C at 12000g for 10 minutes, take the supernatant on ice before testing.
3. **Serum (plasma) or other liquids sample:** Take 100 $\mu$ L liquid sample and add 1mL Extraction reagent I, centrifuge at 4°C at 12000g for 10min, take 0.8mL supernatant, and then add 0.15mL Extraction reagent II, fully shake and mix, centrifuge at 4°C at 12000g for 10min, take the supernatant on ice before testing.

### II. Determination

1. Preheat the spectrophotometer for more than 30 minutes, adjust the wavelength to 450 nm and set spectrophotometer counter to zero with distilled water.
2. Sample Test (add Reagent in the EP tube):

Reagent ( $\mu$ L)	Blank tube (B)	Standard tube (S)	Test tube (T)	Control tube(C)
Sample	-	-	100	100
Standard goods	-	100	-	-
distilled water	100	-	-	-
Reagent I	265	265	265	400
Reagent II	200	200	200	200
Reagent III	300	300	300	300
Reagent IV	135	135	135	-

Mix thoroughly, keep it at 37°C for 30 minutes (Light avoidance), read the absorbance of wavelength at 450 nm. Note the light absorption values of blank tube, standard tube, test tube and control tube as  $A_B$ ,  $A_S$ ,  $A_T$  and  $A_C$  respectively. Calculation  $\Delta A_T = A_T - A_C$ ;  $\Delta A_S = A_S - A_B$ . The standard tube and blank tube only need to be measured 1-2 times.

### III. Calculation of D-lactic acid content:

1. Protein concentration:

Malic acid content ( $\mu\text{mol}/\text{mg prot}$ ) =  $\Delta A_T \times C_S \div \Delta A_S \times V_S \div (V_S \times C_{pr}) \times F = 0.4 \times \Delta A_T \div \Delta A_S \div C_{pr} \times F$

2. Sample weight:

Malic acid content ( $\mu\text{mol}/\text{g weight}$ ) =  $\Delta A_T \times C_S \div \Delta A_S \times (V_{SV} + V_{EVII}) \div (W \times V_{SV} \div V_{EVI}) \times F$   
 $= 0.475 \times \Delta A_T \div \Delta A_S \div W \times F$

3. Cell amount:

Malic acid content ( $\mu\text{mol}/10^6 \text{ cell}$ ) =  $\Delta A_T \times C_S \div \Delta A_S \times (V_{SV} + V_{EVII}) \div (N \times V_{SV} \div V_{EVI}) \times F$   
 $= 0.475 \times \Delta A_T \div \Delta A_S \div N \times F$

4. Volume of liquid:

Malic acid content ( $\mu\text{mol}/\text{mL}$ ) =  $\Delta A_T \times C_S \div \Delta A_S \times (V_{SV} + V_{EVII}) \div [V_{LS} \times V_{SV} \div (V_{EVI} + V_{LS})] \times F$   
 $= 5.225 \times \Delta A_T \div \Delta A_S \times F$

$C_S$ : Standard tube concentration,  $0.4 \mu\text{mol}/\text{mL}$ ;

$V_S$ : Sample volume,  $0.1 \text{ mL}$ .

$V_{SV}$ : supernatant volume,  $0.8 \text{ mL}$ .

$V_{EVI}$ : Extraction volume I,  $1 \text{ mL}$ ;

$V_{EVII}$ : Extraction volume II,  $0.15 \text{ mL}$ ;

$V_{LS}$ : Liquid sample volume,  $0.1 \text{ mL}$ .

**Note:**

1. Extraction reagent I contains a protein precipitator, so the supernatant cannot be used for protein concentration determination. For determination of protein content, separate tissue should be taken.
2. The determination range of  $\Delta A$  is between  $0.01 - 1$ . If the absorbance value exceeds the linear range, the sample can be diluted with distilled water and then measured again. If the absorbance value is less than the linear range, the sample size needs to be increased and then measured again. Pay attention to the synchronous calculation formula.

**Experimental examples:**

1. Take  $0.1075 \text{ g}$  of Banana pulp was added with  $1 \text{ mL}$  extraction reagent I, homogenized in ice bath, centrifuged,  $0.8 \text{ mL}$  supernatant was added with  $0.15 \text{ mL}$  extraction reagent II, Centrifugally take supernatant and dilute it with distilled water for 8 times, then follow the determination procedure. After determination with  $1 \text{ mL}$  glass cuvette, calculate  $\Delta A_T = A_T - A_C = 0.850 - 0.086 = 0.764$ .  $\Delta A_S = A_S - A_B = 0.570 - 0.093 = 0.477$ , To calculate:  
 Malic acid content ( $\mu\text{mol}/\text{g weight}$ ) =  $0.475 \times \Delta A_T \div \Delta A_S \div W \times F = 56.6174 \mu\text{mol}/\text{g weight}$
2. Take  $0.1033 \text{ g}$  of rabbit muscle was added with  $1 \text{ mL}$  extraction reagent I, homogenized in ice bath, centrifuged,  $0.8 \text{ mL}$  supernatant was added with  $0.15 \text{ mL}$  extraction reagent II, centrifuged and the supernatant was taken according to the measurement procedure. After determination with  $1 \text{ mL}$  glass cuvette, calculate  $\Delta A_T = A_T - A_C = 0.313 - 0.097 = 0.216$ .  $\Delta A_S = A_S - A_B = 0.570 - 0.093 = 0.477$ , To calculate:

Malic acid content ( $\mu\text{mol/g weight}$ ) =  $0.475 \times \Delta A_T \div \Delta A_S \div W \times F = 2.0822 \mu\text{mol/g weight}$

3. Take 100 $\mu\text{L}$  bovine serum and add 1mL extract solution I, centrifuge it, take 0.8mL supernatant and add 0.15mL extract solution II, centrifuge supernatant, and then follow the measurement procedure, After determination with 1 mL glass cuvette, calculate  $\Delta A_T = A_T - A_C = 0.147 - 0.116 = 0.031$ .  $\Delta A_S = A_S - A_B = 0.570 - 0.093 = 0.477$ , To calculate:

Malic acid content ( $\mu\text{mol/mL}$ ) =  $5.225 \times \Delta A_T \div \Delta A_S \times F = 0.3395 \mu\text{mol/mL}$

#### **Related Products:**

BC0710/BC0715	$\alpha$ -Ketoglutarate Dehydrogenase ( $\alpha$ -KGDH) Assay Kit
BC0950/BC0955	Succinate dehydrogenase (SDH) Assay Kit
BC0380/BC0385	Pyruvate dehydrogenase, PDH Assay Kit
BC1060/BC1065	Citrate Synthase(CS) Assay Kit
BC2200/BC2205	Pyruvate(PA) Content Assay Kit