Malic Acid Content Assay Kit

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

Operation Equipment: Spectrophotometer/ Microplate reader

Catalog Number: BC5495

Size:100T/48S

Components:

Extraction reagent I: Liquid 60 mL×1. Store at 2-8℃.

Extraction reagent II: Liquid 10 mL×1. Store at 2-8℃

Reagent I: Liquid 8 mL×1. Store at 2-8℃.

Reagent II: Powder ×1. Store at -20℃, Dissolve with 5mL of distilled water before use. It can be stored at 2-8℃ for 4 weeks.

Reagent III: Liquid 8 mL×1. Store at 2-8℃.

Reagent IV: Liquid 20 μL×1. Store at 2-8℃. Before use according to Reagent IV: Reagent IV diluent =10μL: 1mL (about 40T) proportion dilution Reagent IV standby.

Reagent IV diluent: Liquid 3 mL×1. Store at 2-8℃.

Standard goods: Liquid 1 mL×1. Store at 2-8℃. 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 transmitochondrial membrane transport process of oxidative phosphorylation. In lower organisms, malic acid is converted to lactic acid in the process of malolactic fermentation, which also produces CO2. 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 NH4+. 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/Microplate reader, mortar/homogenizer/cell ultrasonic crusher, centrifuge, constant temperature foster box/water-bath, centrifuge, micro quartz cuvette/96 well flat-bottom plate, 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℃. Take 0.8mL of supernatant, add 0.15mL of Extraction reagent II fully shake and mix, centrifuge at 4℃ 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℃. Take 0.8mL of supernatant, add 0.15mL of Extraction reagent II fully shake and mix, centrifuge at 4℃ at 12000g for 10 minutes, take the supernatant on ice before testing.
3. **Serum (plasma) or other liquids sample:** Take 100μL liquid sample and add 1mL Extraction reagent I, centrifuge at 4℃ at 12000g for 10min, take 0.8mL supernatant, and then add 0.15mL Extraction reagent II, fully shake and mix, centrifuge at 4℃ at 12000g for 10min, take the supernatant on ice before testing.

II. Determination

1. Preheat the spectrophotometer or microplate reader 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/96 well flat-bottom plate):

Reagent（μL）	Blank tube (B)	Standard tube (S)	Test tube (T)	Control tube(C)
Sample	-	-	20	20
Standard goods	-	20	-	-
distilled water	20	-	-	-
Reagent I	55	55	55	80
Reagent II	40	40	40	40
Reagent III	60	60	60	60
Reagent IV	25	25	25	-

Mix thoroughly, keep it at 37℃ 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 Ac respectively. Calculation $\Delta A_T = A_T - Ac$; $\Delta A_S = As - A_B$. The standard tube and blank tube only need to be measured 1-2 times.

III. Calculation of malic acid content:

1. Sample weight:
   Malic acid content（μmol/g weight）= $\Delta A_T \times C_S \div \Delta A_S \times (V_{SV}+V_{EVII}) \div (W \times V_{SV} \div V_{EVI}) \times F$
   $= 0.475 \times \Delta A_T \div \Delta A_S \div W \times F$
2. Protein concentration:
   Malic acid content（μmol/mg prot）= $\Delta A_T \times C_S \div \Delta A_S \times V_S \div (V_S \times Cpr) \times F = 0.4 \times \Delta A_T \div \Delta A_S \div Cpr \times F$
3. Cell amount:
   Malic acid content（μmol/10^6 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（μmol/mL）= $\Delta A_T \times C_S \div (\Delta A_S \div C_S) \times (V_{SV}+V_{EVII}) \div [V_{LS} \times V_{SV} \div (V_{EVI+} V_{LS})]$
   $= 4.082 \times \Delta A_T \div \Delta A_S$

C_S: Standard tube concentration, 0.3125μmol/mL;
V_S: Sample volume,0.1 mL.
V_{SV}: supernatant volume,0.8 mL.
V_{EVI}: Extraction volume I, 1 mL;
V_{EVII}: Extraction volume II, 0.15 mL;
V_{LS}: Liquid sample volume,0.1 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 ΔA 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 0.1075g of Banana pulp was added with 1mL extraction reagent I, homogenized in ice bath, centrifuged, 0.8mL supernatant was added with 0.15mL extraction reagent II, Centrifugally take supernatant and dilute it with distilled water for 8 times, then follow the determination procedure. After determination with 96 well flat-bottom plate, calculate $\Delta A_T = A_T - A_C = 0.581-0.098 = 0.483$. $\Delta A_S = A_S - A_B = 0.409-0.101 = 0.308$, To calculate:
   Malic acid content（μmol/g weight）$= 0.475 \times \Delta A_T \div \Delta A_S \div W \times F = 55.43$μmol/g weight
2. Take 0.1033g of rabbit liver was added with 1mL extraction reagent I, homogenized in ice bath, centrifuged, 0.8mL supernatant was added with 0.15mL extraction reagent II, centrifuged and the supernatant was taken according to the measurement procedure. After determination with 96 well

flat-bottom plate, calculate $\Delta A_T=A_T - A_C$ =0.271-0.118=0.153. $\Delta A_S = A_S - A_B$ =0.409-0.101=0.308, To calculate:

Malic acid content（μmol/g weight）=$0.475\times\Delta A_T\div\Delta A_S\div W\times F$= 2.28μmol/g weight

Related Products：

BC0710/BC0715	α-Ketoglutarate Dehydrogenase (α-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