Malic Acid Content Assay Kit (WST colorimetry)

Note: The reagents have been changed, please be aware of and follow this instruction strictly.

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. Reagents may be dispensed and stored at 20℃ for 4 weeks, avoiding repeated freezing and thawing;

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

Reagent IV: Powder ×1. Store at -20℃, Before use, add 80μL of Reagent IV dilution solution to dissolve (can be shaken to promote dissolution), can be dispensed and stored at 20 ℃ for 4 weeks, avoid repeated freezing and thawing;

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

Reagent IV working solution: Before use, dilute Reagent IV according to the ratio of Reagent IV: Reagent IV Diluent = 10 μL: 1mL (about 40T) and standby, ready to use;

Standard: Liquid 1 mL×1. Store at 2-8℃. 100μmol/mL malic acid Standard solution.

0.4 μmol/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 CO_2. 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_4^+. 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:**

   Accordance ratio weight(g): Extract solution I(mL)=1: 5~10. (Suggested 0.1g tissue with 1mL Extract solution I). Homogenate on ice bath. 12000 g centrifuge for 10 min at 4℃. Add 0.15mL Extract solution II slowly to 0.8 mL supernatant. Blend slowly until no bubbles. Then 12000 g centrifuge for 10 min at 4℃. Supernatant is for test.

2. **Cells:**

   Accordance ratio cell amount (10^4): Extract solution I(mL)=500~1000:1. (Suggested 5 million cells with 1mL Extract solution I). Breaking cells (300W, work time 3s, interval 7s for 3 min) by ultrasonic on ice bath. 12000 g centrifuge for 10 min at 4℃. Add 0.15 mL Extract solution II to 0.8mL supernatant slowly. Blend slowly until no bubbles. 12000 g centrifuge for 10 min at 4℃. Supernatant is used for test.

3. **Serum (plasma) sample:**

   Add 1 mL Extract solution I to 100 μL serum(plasma). 12000 g centrifuge for 10 min at 4℃. Add 0.15 mL Extract solution II to 0.8 mL supernatant slowly. Blend slowly until no bubbles. Centrifuge for 10 min at 12000 g. Supernatant is used for test.

   Note: Extract solution II needs to be added slowly, and a large number of bubbles will be generated after addition, so it is recommended to use a 2mL EP tube for the operation.

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 $\Delta A_T = A_T - Ac$; $\Delta A_S = A_S - A_B$. 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）$= \Delta A_T \times C_S \div (\Delta A_S \div C_S) \times (V_{SV}+V_{EVII}) \div [V_{LS} \times V_{SV} \div (V_{EVI+} V_{LS})]$
   $= 5.225 \times \Delta A_T \div \Delta A_S$

C_S: Standard tube concentration,0.4μmol/mL;
V_S: Sample volume,0.02 mL.
V_{SV}: supernatant volume,0.8 mL.
V_{EVI}: Extraction volume I, 1 mL;
V_{EVII}: Extraction volume II, 0.15 mL;
N: Number of cells, in millions;
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）=$0.475\times\Delta A_T\div\Delta A_S\div W\times F$=55.43μmol/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

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