

Isocitric Acid Content Assay Kit

Detection Equipment: Spectrophotometer

Catalog Number: BC5950

Size: 50T/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 I	Liquid 60 mL×1	2-8°C storage
Extract II	Liquid 10 mL×1	2-8°C storage
Reagent I	Liquid 60 mL×1	2-8°C storage
Reagent II	Liquid 750μL×1	2-8°C storage
Reagent III	Powder ×1	2-8°C storage
Powder I	Powder ×1	2-8°C storage
Standard	Powder ×1	2-8°C storage

Solution reparation :

1. Reagent II working liquid: Centrifuge before use, according to the sample size according to Reagent II: Distilled water =10μL: 90μL (a total of 100μL, about 1T) the proportion of preparation and use, now used.
2. Reagent III: Before use, add 4 mL distilled water to fully dissolve, and storage the unused reagent for 4 weeks at -20°C to avoid repeated freezing and thawing.
3. Standard: before use, add 1 mL distilled water to fully dissolve into 20μmol/mL isocitric acid standard.
4. Working liquid: Before clinical use according to the sample size according to Reagent I: Reagent II working liquid: Reagent III =750μL: 100μL: 50μL (a total of 900μL, about 1T) into the working liquid, now used.

Description:

Isocitric Acid, an isomer of citric acid, is found in large amounts in many fruits and vegetables and in foods made from these ingredients. What organisms can use is type D isocitric acid, a component of the tricarboxylic acid cycle.

Isocitric acid is converted to α -ketoglutaric acid by isocitric dehydrogenase (ICDH, EC1.1.1.41), while NAD (P) is reduced to NAD (P) H, from which isocitric acid content can be calculated.



Note: Before the experiment, it is recommended to select 2-3 sample with large expected differences for pre-experiment. If the absorption 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, low temperature centrifuge, analytical balance, water bath/constant temperature incubator, 1mL quartz colorimetric dish, adjustable pipette, 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.Organization: According to mass (g) : Extract I volume (mL) = 1: 5 ~10 (it is recommended to weigh about 0.1g and add 1mL of Extract I), add Extract I , centrifuge at 4°C for 12000g for 10min after ice bath homogenization, take 0.8mL of supernatant, slowly add 0.15mL of Extract II, slowly blow and mix until no bubbles emerge, centrifuge at 4°C for 12000g for 10min, take supernatant to be measured.**(The extraction solution II needs to be added slowly, and a large number of bubbles will be generated after addition. It is recommended to use 2mL EP tube for operation.)**

2.Bacteria or cells: Collect bacteria or cells into the centrifuge tube and discard the supernatant after centrifugation; According to the number of bacteria or cells (10^6) : the proportion of extraction liquid volume (mL) = 5~ 10:1 (it is recommended that 5×10^6 cells be added to 1mL of extraction liquid 1), the cells are broken by ice bath ultrasound (power 300w, ultrasound 3 s, interval 7 s, total time 3min); Centrifuge at 4°C for 12000g for 10min, take 0.8mL supernatant, slowly add 0.15mL extract solution 2, slowly blow and mix until no bubbles emerge, centrifuge at 4°C for 12000g for 10min, take supernatant to be measured.**(The extraction solution II needs to be added slowly, and a large number of bubbles will be generated after addition. It is recommended to use 2mL EP tube for operation.)**

3.Juice and other liquids: Take 500 μ L liquid sample, add 1mg powder and mix well, boil water for 5min (wrap the sealing film to prevent bursting), let it stand at room temperature, centrifuge at 4°C 12000g for 10min, and take the supernatant to be measured.

II. Measurement Steps

- The ultraviolet spectrophotometer was preheated for more than 30min, the wavelength was adjusted to 340nm, and the distilled water was zeroed.
- Preheat Reagent IV at 37°C for 10min.
- Dilution of standard: 20 μ mol/mL isocitric acid standard is diluted with distilled water to obtain 8, 4, 2, 1, 0.5 μ mol/mL standard for use.
- Standard dilution table:

Serial number	Concentration before dilution (μ mol/mL)	Standard volume (μ L)	Distilled water volume (μ L)	Diluted concentration (μ mol/mL)
1	20	200	300	8
2	8	250	250	4
3	4	250	250	2
4	2	250	250	1
5	1	250	250	0.5

Note: 100 μ L of standard material is required for each standard tube in the following experiment (be careful not to test the absorbance of the standard material directly in this step).

5. Sample determination (add the following reagents in 1mL quartz colorimetric dish)

Reagent name (μ L)	Test tube	Standard tube	Blank tube
Sample	100	-	-
Standard	-	100	-
Distilled water	-	-	100
Working liquid	900	900	900

Thoroughly mixed, immediately determine the absorbance A1 at 10s at 340nm, and then quickly react at 37°C for 10min to determine the absorbance A2 at 10min10s, respectively recorded as A1_{text}, A2_{text}, A1_{standard}, A2_{standard}, A1_{blank}, A2_{blank}. Calculate $\Delta A_{\text{standard}} = (A2_{\text{standard}} - A1_{\text{standard}}) - (A2_{\text{blank}} - A1_{\text{blank}})$, $\Delta A_{\text{text}} = (A2_{\text{text}} - A1_{\text{text}}) - (A2_{\text{blank}} - A1_{\text{blank}})$. Each standard tube and blank tube only need to be tested 1-2 times.

III. Calculations

1. Drawing of standard curve

The standard curve is established according to the concentration of the standard tube (x, μ mol/mL) and the absorbance $\Delta A_{\text{standard}}$ (y, $\Delta A_{\text{standard}}$). According to the standard curve, the ΔA_{text} (y, ΔA_{text}) is brought into the formula to calculate the sample concentration (x, μ mol/mL).

2. Isocitric acid content calculation:

1) Calculated by sample protein concentration:

$$\text{Isocitric acid content } (\mu\text{mol/g prot}) = x \times V_{\text{sample}} \div (Cpr \times V_{\text{sample}}) \times F = x \div Cpr \times F$$

2) Calculated by sample quality:

$$\text{Isocitric acid content } (\mu\text{mol/g mass}) = x \times (V_{\text{supernatant}} + V_{\text{Extract II}}) \div (W \times V_{\text{supernatant}} \div V_{\text{Extract I}}) \times F = 1.1875 \times x \div W \times F$$

3) Calculated by the number of bacteria or cells:

$$\text{Isocitric acid content } (\mu\text{mol}/10^6 \text{ cell}) = x \times (V_{\text{supernatant}} + V_{\text{Extract II}}) \div (N \times V_{\text{supernatant}} \div V_{\text{Extract I}}) \times F = 1.1875 \times x \div N \times F$$

4) Calculated by liquid volume:

$$\text{Isocitric acid content } (\mu\text{mol/mL}) = x \times F$$

V_{sample}: Added sample volume, 0.1mL;

V_{supernatant}: Liquid volume of supernatant during extraction, 0.8mL;

V_{Extract II}: The volume of Extract II added, 0.15mL;

V_{Extract I}: Volume of added Extract I, 1mL;

Cpr: Sample protein concentration, mg/mL;

W: Sample quality, g;

N: Number of cells, in millions;

F: Dilution ratio.

Note:

1. 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.
2. If the sample $\Delta A < 0.005$, the sample size can be appropriately increased after determination; If the sample $\Delta A > 1.2$ or $A_{text} > 1.5$, can be measured after diluting the supernatant with distilled water.

Note that the dilution factor in the calculation formula is changed simultaneously.

3. Extract I contains protein precipitator, so the supernatant can not be used for protein concentration determination, if you need to determine protein concentration, need to take another tissue.

Experimental example:

1. Take 0.1139g apple and add it into the extraction solution for ice bath homogenization. After taking the supernub, follow the determination steps and use 1mL quartz colorimetric plate to measure $\Delta A_{text} = (A2_{text} - A1_{text}) - (A2_{blank} - A1_{blank}) = (0.241 - 0.125) - (0.066 - 0.059) = 0.109$. Bring in the standard curve $y = 0.1414x - 0.0466$, $R^2 = 0.9991$, calculate $x = 1.100$, calculate the isocitric acid content according to the sample mass, and get:

Isocitric acid content ($\mu\text{mol/g mass}$) = $1.1875 \times x \div W \times F = 11.468 \mu\text{mol/g mass}$ 。

2. Take 0.1033g orange leaves and add them into the extract for ice bath homogenization, take the supernant and dilute it by 2 times with distilled water, follow the measurement procedure, and use 1mL quartz colorimetric plate to measure $\Delta A_{text} = (A2_{text} - A1_{text}) - (A2_{blank} - A1_{blank}) = (1.001 - 0.923) - (0.066 - 0.059) = 0.071$. Bring in the standard curve $y = 0.1414x - 0.0466$, $R^2 = 0.9991$, calculate $x = 0.832$, calculate the isocitric acid content according to the sample mass, and get:

Isocitric acid content ($\mu\text{mol/g mass}$) = $1.1875 \times x \div W \times F = 19.129 \mu\text{mol/g mass}$ 。

3. Take 500 μL orange juice beverage and take supernant according to the measurement procedure, and use 1mL quartz colorimetric plate to measure $\Delta A_{text} = (A2_{text} - A1_{text}) - (A2_{blank} - A1_{blank}) = (0.856 - 0.840) - (0.066 - 0.059) = 0.009$, Bring in the standard curve $y = 0.1414x - 0.0466$, $R^2 = 0.9991$, calculate $x = 0.393$, calculate the isocitric acid content according to the volume of liquid:

Isocitric acid content ($\mu\text{mol/mL}$) = $x \times F = 0.393 \mu\text{mol/mL}$ 。

References:

[1] Kvasni Ka, Franti Ek, et al. "Determination of Isocitric Acid in Citrus Juice—A Comparison of HPLC, Enzyme Set and Capillary Isotachopheresis Methods." *Journal of Food Composition & Analysis* 15.6(2002):685-691.

[2] Kong, Min Jung, et al. "Mitochondrial NADP⁺-dependent isocitrate dehydrogenase deficiency increases cisplatin-induced oxidative damage in the kidney tubule cells." *Cell Death & Disease* 9.5(2018):488.

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- BC2150/BC2155 柠檬酸 (CA) 含量检测试剂盒
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