Isocitric Acid Content Assay Kit

Detection Equipment: Spectrophotometer/microplate reader

Catalog Number: BC5955

Size: 100T/96S

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 110 mL×1	2-8℃ storage
Extract II	Liquid 20 mL×1	2-8℃ storage
Reagent I	Liquid 20 mL×1	2-8℃ storage
Reagent II	Liquid 250μL×1	2-8℃ storage
Reagent III	Powder ×1	2-8℃ storage
Powder I	Powder ×1	2-8℃ storage
Standard	Powder ×1	2-8℃ storage

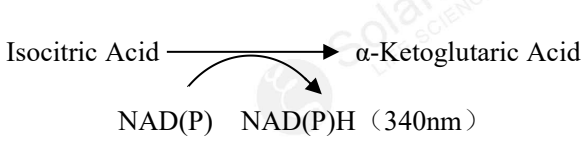
Solution reparation :

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 5T) the proportion of preparation and use, now used.
2. Reagent III: Before use, add 1.3 mL distilled water to fully dissolve, and storage the unused reagent for 4 weeks at -20℃ 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 =150μL: 20μL: 10μL (a total of 180μL, 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 A-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/microplate reader, low temperature centrifuge, analytical balance, water bath/constant temperature incubator, micro glass cuvette/96 well flat-bottom plate, 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℃ 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℃ 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℃ 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℃ 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 500μL 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℃ 12000g for 10min, and take the supernatant to be measured.

II. Measurement Steps

1. The ultraviolet spectrophotometer/microplate reader was preheated for more than 30min, the wavelength was adjusted to 340nm, and the distilled water was zeroed.
2. Preheat Reagent IV at 37℃ for 10min.
3. Dilution of standard: 20μmol/mL isocitric acid standard is diluted with distilled water to obtain 12, 10, 8, 4, 2, 1, 0.5μmol/mL standard for use.
4. Standard dilution table:

Serial number	Concentration before dilution (μmol/mL)	Standard volume (μL)	Distilled water volume (μL)	Diluted concentration (μmol/mL)
1	20	300	200	12
2	20	300	300	10
3	10	400	100	8
4	8	200	200	4
5	4	200	200	2

6	2	200	200	1
7	1	200	200	0.5

Note: 20μL 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 micro glass cuvette/96 well flat-bottom plate)

Reagent name（μL）	Test tube	Standard tube	Blank tube
Sample	20	-	-
Standard	-	20	-
Distilled water	-	-	20
Working liquid	180	180	180
Thoroughly mixed, immediately determine the absorbance A1 at 10s at 340nm, and then quickly react at 37℃ for 10min (The temperature control function of the enzyme marker can adjust the temperature to 37℃)to determine the absorbance A2 at 10min10s, respectively recorded as $A1_{text}$, $A2_{text}$, $A1_{standard}$, $A2_{standard}$, $A1_{blank}$, $A2_{blank}$. Calculate $\Delta A_{standard} = (A2_{standard} - A1_{standard}) - (A2_{blank} - A1_{blank})$, $\Delta A_{text} = (A2_{text} - A1_{text}) - (A2_{blank} - A1_{blank})$. 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, μmol/mL) and the absorbance $\Delta A_{standard}$ (y, $\Delta A_{standard}$). According to the standard curve, the ΔA_{text}(y, ΔA_{text}) is brought into the formula to calculate the sample concentration (x, μmol/mL).

2. Isocitric acid content calculation:

1) Calculated by sample protein concentration:

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

2) Calculated by sample quality:

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

3) Calculated by the number of bacteria or cells:

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

4) Calculated by liquid volume:

Isocitric acid content（μmol/mL）$=x \times F$

V_{sample}: Added sample volume, 0.02mL;

$V_{supernatant}$: 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 ΔA<0.005, the sample size can be appropriately increased after determination; If the sample ΔA>1.0 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 96-well UV plate to measure ΔA_{text} = ($A2_{text}$ -$A1_{text}$) - ($A2_{blank}$ -$A1_{blank}$) =（0.140-0.089）-（0.060-0.058）=0.049. Bring in the standard curve y=0.0658x-0.0178, R^2=0.9978, calculate x=1.015, calculate the isocitric acid content according to the sample mass, and get:
   Isocitric acid content（μmol/g mass）=1.1875×x÷W×F=10.582 μmol/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 96-well UV plate to measure ΔA_{text} = ($A2_{text}$ -$A1_{text}$) - ($A2_{blank}$ -$A1_{blank}$) =（1.445-1.415）-（0.060-0.058）=0.028. Bring in the standard curve y=0.0658x-0.0178, R^2=0.9978, calculate x=0.696, calculate the isocitric acid content according to the sample mass, and get:
   Isocitric acid content（μmol/g mass）=1.1875×x÷W×F=16.002 μmol/g mass。
3. Take 500μL orange juice beverage and take supernant according to the measurement procedure, and use 96-well UV plate to measure ΔA_{text} = ($A2_{text}$ -$A1_{text}$) - ($A2_{blank}$ -A1 blank) =（0.523-0.516）-（0.060-0.058）=0.005, Bring in the standard curve y=0.0658x-0.0178, R^2=0.9978, calculate x=0.347, calculate the isocitric acid content according to the volume of liquid:
   Isocitric acid content（μmol/mL）=x×F=0.347 μmol/mL。

References：

[1] Kvasni Ka, Franti Ek, et al. "Determination of Isocitric Acid in Citrus Juice—A Comparison of HPLC, Enzyme Set and Capillary Isotachophoresis 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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