Mitochondrial Isocitrate Dehydrogenase (ICDHm) Activity Colorimetric Assay Kit

Cat #: orb1173191 (manual)

Product name: Mitochondrial Isocitrate Dehydrogenase (ICDHm) Activity Colorimetric Assay Kit

Catalog number: orb1173191

Applicable samples: Animal and Plant Tissues, Cells

Storage: Stored at -20℃ for 6 months, protected from light

Assay Principle

Mitochondrial Isocitrate Dehydrogenase (ICDHm) is widely presented in mitochondria of animals, plants, microorganisms, and cultured cells. It can catalyze Isocitrate decarboxylation and produce α-Ketoglutarate during Kreb's cycle, and reduce $NADP^+$ to NADPH. Micro Mitochondrial Isocitrate Dehydrogenase (ICDHm) Assay Kit provides a simple, convenient, and rapid method for detecting isocitrate dehydrogenase activity, which is suitable for the detection of animal tissues, plant tissues, cells. The principle is that ICDHm can catalyze $NADP^+$ reduction to produce NADPH, NADPH has an absorption peak at 340 nm, and ICDHM activity can be calculated by the change of light absorption at 340 nm.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	48 T	96 T	
Extraction Buffer	50 mL	100 mL	4℃
Reagent I	10 mL	20 mL	4℃
Reagent II	0.75 mL	1.5 mL	-20℃, protected from light
Reagent III	9 mL	18 mL	4℃
Reagent IV	1	1	4℃
Reagent V	1	1	-20℃, protected from light

Materials Required but Not Supplied

- Microplate reader or ultraviolet spectrophotometer capable of measuring absorbance at 340 nm
- Ice maker, refrigerated centrifuge
- Water bath
- 96-well UV plate or microquartz cuvette
- Precision pipettes, disposable pipette tips
- Deionized water
- Dounce homogenizer (for tissue samples)

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Reagent Preparation

Extraction Buffer: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃.

Reagent I: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃.

Reagent II: Ready to use as supplied. Equilibrate to room temperature before use. Store at -20℃, protected from light.

Reagent III: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃.

Working Solution: Prepare before use, resolve Reagent IV with Reagent III, mix well and store at 4℃.

Working Reagent V: Prepare before use, resolve Reagent V with 0.5 mL deionized water while using 48 T kit; Resolve Reagent V with 1 mL deionized water while using 96 T kit; The remaining reagents should be stored at -20℃ and protected from light after aliquoting to avoid repeated freezing and thawing.

Sample Preparation

Note: Fresh samples are recommended, If not assayed immediately, samples can be stored at -80 ℃ for one month. Processed samples must be assayed immediately. All samples and reagents should be on ice to avoid denaturation and

deactivation.

Extraction of cytoplasmic protein and mitochondrial protein from cells and tissue:

1. Weigh 0.1 g tissue or collect 5×10^6 cells, add 1 mL Extraction Buffer and 10 µL Reagent II, homogenize on ice. Centrifuge at 600 g for 5 min at 4℃. Collect the supernatant to a new centrifuge tube and discard the pellet.
2. Centrifuge the supernatant again at 11,000 g for 10 min at 4℃, and obtain the supernatant and precipitate respectively.
3. (Optional) The supernatant collected in step 2 is cytoplasmic extract, which can be used to determine ICDHm leaking from mitochondria.
4. Add 200 µL Reagent I and 2 µL Reagent II to the precipitate collected in step 2, resuspend the precipitate sufficiently, and use it to detect the activity of ICDHm in the next step.

Assay Procedure

1. Preheat the microplate reader or ultraviolet spectrophotometer for more than 30 min, and adjust the wavelength to 340 nm. Ultraviolet spectrophotometer was returned to zero with deionized water.
2. Incubate Working Solution for 10 min at 37°C (mammal) or 25°C (other species).
3. Add 10 µL sample, 180 µL Working Solution, and then 10 µL Working Reagent V in a 96-well UV plate or microquartz cuvette. After mixing quickly, record the absorbance values of 20 s and 2 min 20 s at 340 nm with a microplate reader, mark as A1 and A2, and calculate ΔA=A2-A1.

Note: In order to guarantee the accuracy of experimental results, need to do a pre-experiment with 2-3 samples. If ΔA is less than 0.01, increase the sample quantity appropriately. If ΔA is greater than 0.3, the sample can be appropriately diluted with Extraction Buffer, the calculated result multiplied by the dilution factor, or decrease the sample quantity appropriately.

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Data Analysis

Note: We provide you with calculation formulae, including the derivation process and final formula. The two are exactly equal. It is suggested that the concise calculation formula in bold is final formula.

A. 96-well UV plates calculation formula

1. Calculated by fresh weight of samples:

Unit definition: One enzyme activity unit defines as 1 nmol NADPH produced by 1 g tissue per minute in the reaction system. ICDHmSupernatant activity (U/g fresh weight)=[ΔASupernatant×VTotal÷(ε×d)×10^9]÷(VSample÷VExtraction×W)÷T**=3,247.59×ΔASupernatant÷W**

$ICDHm_{Pellet}$ activity (U/g fresh weight)=[ΔA_{Pellet}×V_{Total}÷(ε×d)×10^9]÷(V_{Sample}÷$V_{Total\ Sample}$×W)÷T**=649.52×ΔA_{Pellet}÷W**

Total ICDHm activity (U/g fresh weight)=$ICDHm_{Supernatant}$ activity+$ICDHm_{Pellet}$ activity**=3,247.59×$\Delta A_{Supernatant}$÷W+649.52×ΔA_{Pellet} ÷W**

2. Calculated by cell density:

Unit definition: One enzyme activity unit defines as 1 nmol NADPH produced by 10^4 cells per minute in the reaction system. ICDHm activity (U/10^4 cells)=[ΔA_{Test}×V_{Total}÷(ε×d)×10^9]÷(V_{Sample}÷$V_{Total\ Sample}$×500)÷T**=1.3×ΔA_{Test}**

Where: V_{Total}: total reaction volume, 2×10^{-4} L; ε: NADPH molar extinction coefficient, 6.22×10^3 mol/L/cm; d: 0.5 cm; V_{Sample}: sample volume added, 0.01 mL; T: reaction time, 2 min; $\Delta A_{Supernatant}$: OD value of supernatant; $V_{Extraction}$: sample extract volume, 1.01 mL; W: sample weight, g; ΔA_{Pellet}: OD value of pellet; $V_{Total\ Sample}$: the volume of adding Reagent I and II, 0.202 mL; 500: total number of cells, 5×10^6.

B. Microquartz cuvette calculation formula

The optical diameter d: 0.5 cm in the above calculation formula can be adjusted to d: 1 cm for calculation.

Precautions

1. It is not suggested to test too many samples at the same time, because enzyme activity is calculated by the variation of absorbance value per unit time.

Disclaimer

The reagent is only used in the field of scientific research, not suitable for clinical diagnosis or other purposes.

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