Isocitrate Lyase (ICL) Activity Assay Kit

Note: Take two or three different samples for prediction before test.

Operation Equipment: Spectrophotometer/ Microplate reader

Cat No: NA0482

Size:100T/96S

Components:

Extract solution: Liquid100 mL×1. Storage at 4℃. Add reagent VII to the extract solution before use.

Reagent I: 3 mL×1. Storage at 4℃.

Reagent II: 4 mL×1. Storage at 4℃.

Reagent III: Powder×1. Storage at -20℃. Dissolve it thoroughly with 5mL of distilled water before use. The reagents can be stored at - 20℃ after being packed separately. Avoid repeated freezing and thawing.

Reagent IV: Powder×1. Storage at -20℃. Dissolve it thoroughly with 5mL of distilled water before use. The reagents can be stored at - 20℃ after being packed separately. Avoid repeated freezing and thawing.

Reagent V: Liquid 40 μL×1. Storage at 4℃. The liquid is placed in the EP tube in the reagent bottle. According to the dosage and the volume ratio of Reagent V: distilled water=1:8, and the mixture is ready to use.

Reagent VI: Powder×1. Storage at 4℃. Dissolve it thoroughly with 5 mL of distilled water before use. The reagents can be stored at 4℃.

Reagent VII: Powder×1. Storage at 4℃.

Product Description:

ICL (ec4.1.3.1) mainly exists in plants and microorganisms. During the germination process of oil seeds, fat is converted into carbohydrate through glyoxylate cycle and other processes. ICL is one of the key enzymes in glyoxylate cycle.

The degradation of isocitric acid to glyoxylic acid and succinic acid is catalyzed by ICL. Glyoxylic acid and NADH generate ethanol and NAD under the action of LDH. NADH has a characteristic absorption peak at 340nm. Monitoring the reduction rate of 340nm absorbance can indirectly reflect the activity of ICL.

Reagents and Equipment Required but Not Provided:

Spectrophotometer/ Microplate reader, water bath, desk centrifuge, adjustable transferpettor, micro quartz cuvette/ 96 well flat-bottom plate, mortar / homogenizer, ice and distilled water.

Sample preparation:

1. Cells or bacteria: Collect bacteria or cells into centrifuge tube, after centrifugation discard supernatant. Suggest 2 million of bacteria or cells with 0.4 mL of extract solution, splitting with ultrasonication (ice

bath, power 20%, work time 3s, interval 10s, for 30 times). Centrifuge at 15000 ×g for 10 minutes at 4℃ to remove insoluble materials and take the supernatant on ice before testing.

2. Tissue: Add 1 mL of extract solution into 0.1 g of tissue, fully grinding on ice. Centrifuge at 15000 ×g for 10 minutes at 4℃ to remove insoluble materials and take the supernatant on ice before testing.

Procedure:

1. Preheat spectrophotometer/ microplate reader for 30 minutes, adjust the wavelength to 340nm and set the counter to zero with distilled water.
2. Working solution: Mix the Reagent I, Reagent II, Reagent III, Reagent IV and Reagent V as a ratio of 29:35:42:42:3.
3. Add the following reagents to 1mL glass cuvette:

Reagent	Test tube (T)
Working solution (μL)	151
Sample (μL)	7
reagent VI	42

Add working solution and sample to 1 mL quartz cuvette. Mix thoroughly and timing, measure the absorption at 340 nm at 10s recorded as A1, then put the cuvette and react solution to 37℃(mammal) or 25℃ (other species) water bath for 2 minutes. Take out and dry it quickly, detect the absorbance at 340 nm at 2min 10s, recorded as A2, calculate $\Delta A=\Delta A_1-\Delta A_2$

Calculation:

1. micro quartz cuvette:

1) Tissue:

A. Protein concentration:

Unit definition: One unit of enzyme activity is defined as the amount of enzyme that catalyzes the consumption of 1 nmol NADH per minute every mg of tissue protein in react system.

ICL (U/mg prot) $=(\Delta A\div d\div\varepsilon\times Vrv\times10^9)\div(Cpr\times Vs)\div T=2297\times\Delta A\div Cpr$

B. Sample weight

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the consumption of 1 nmol NADH per minute every gram of tissue in react system.

ICL (U/g weight) $=(\Delta A\div d\div\varepsilon\times Vrv\times10^9)\div(W\div Ve\times Vs)\div T=2297\times\Delta A\div W$

2) Bacteria or cells:

A. Protein concentration:

Unit definition: One unit of enzyme activity is defined as the amount of enzyme that catalyzes the consumption of 1 nmol NADH per minute every mg of tissue protein in react system.

ICL (U/mg prot) $=(\Delta A\div d\div\varepsilon\times Vrv\times10^9)\div(Cpr\times Vs)\div T=2297\times\Delta A\div Cpr$

B. Density of bacteria or cell:

Unit definition: One unit of enzyme activity is defined as the amount of enzyme that catalyze the consumption of 1 nmol NADH per minute every 10^4 bacteria or cells in react system.

ICL (U/10^4 cell)=(ΔA÷d÷ε×Vrv×10^9)÷(500×Vs)÷T=4.59×ΔA

Cpr: Sample concentration (mg/mL);
W: Sample weight(g);
Vs: sample volume (mL), 0.007 mL;
Ve: Extraction solution volume(mL), 1mL;
Vrv: Total reaction volume, 0.0002L;
T: Reaction time (min), 2 minutes;
500: Cells or bacteria amount, 500×10 thousand;
d: Light path, 1 cm;
ε: ICL extinction coefficient, 6.22×10^3 L/mol/cm.

2. 96 well flat-bottom plate

1）Tissue:

A. Protein concentration:

Unit definition: One unit of enzyme activity is defined as the amount of enzyme that catalyzes the consumption of 1 nmol NADH per minute every mg of tissue protein in react system.

ICL (U/mg prot) =(ΔA÷d÷ε×Vrv×10^9) ÷(Cpr×Vs)÷T=3828×ΔA ÷Cpr

B. Sample weight

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the consumption of 1 nmol NADH per minute every gram of tissue in react system.

ICL (U/g weight) =(ΔA÷d÷ε×Vrv×10^9) ÷(W÷Ve×Vs)÷T=3828×ΔA ÷W

2）Bacteria or cells:

C. Protein concentration:

Unit definition: One unit of enzyme activity is defined as the amount of enzyme that catalyzes the consumption of 1 nmol NADH per minute every mg of tissue protein in react system.

ICL (U/mg prot) =(ΔA÷d÷ε×Vrv×10^9) ÷(Cpr×Vs)÷T=3828×ΔA ÷Cpr

D. Density of bacteria or cell:

Unit definition: One unit of enzyme activity is defined as the amount of enzyme that catalyze the consumption of 1 nmol NADH per minute every 10^4 bacteria or cells in react system.

ICL (U/10^4 cell)=(ΔA÷d÷ε×Vrv×10^9)÷(500×Vs)÷T=7.65×ΔA

Cpr: Sample concentration (mg/mL);
W: Sample weight(g);
Vs: sample volume (mL), 0.007 mL;
Ve: Extraction solution volume(mL), 1mL;
Vrv: Total reaction volume, 0.0002L;
T: Reaction time (min), 2 minutes;
500: Cells or bacteria amount, 500×10 thousand;
d: Light path, 0.6 cm;

ε: ICL extinction coefficient, 6.22×10^3 L/mol/cm.

Note:

1. During the determination, the sample and all reagents are placed on ice to avoid denaturation and deactivation.
2. The temperature of the reaction solution in the cuvette must be kept at 37℃ or 25℃. Take a small beaker and put it into a certain amount of 37℃ or 25℃ distilled water. Put the beaker into a 37 ℃ or 25 ℃ water bath. Put the cuvette and reaction solution into the beaker during the reaction.

Recent Product Citations：

[1] Sun J, Jia H, Wang P, et al. Exogenous gibberellin weakens lipid breakdown by increasing soluble sugars levels in early germination of zanthoxylum seeds[J]. Plant science, 2019, 280: 155-163.

References：

[1] Maffei M, Bertea C M, Garneri F, et al. Effect of benzoic acid hydroxy-and methoxy-ring substituents during cucumber (Cucumis sativus L.) germination. I.: Isocitrate lyase and catalase activity[J]. Plant Science, 1999, 141(2): 139-147.

Related Products：

NA0640/NA0377 Acetokinase(ACK) Activity Assay Kit
NA0643/NA0401 Plant Dehydrogenase(PDHA) Activity Assay Kit