Succinate Dehydrogenase (SDH)Activity Assay Kit

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

Detection equipment: Spectrophotometer/Microplate reader

Cat No: NA0558

Size: 100T/96S

Components

Reagent I: 110 mL×1, store at -20℃；

Reagent II: 1 mL×1, store at -20℃；

Reagent III: 18 mL×1, store at 4℃；

Reagent IV: Powder×1, store at 4℃; When the solution will be used, add it into Reagent III to dissolve for use.

Reagent V: Powder×1, store at 4℃; Add 4 mL of distilled water when the solution will be used, the unused reagents are stored at 4℃.

Reagent VI: Powder×1, store at -20℃; Add 3.333 mL of distilled water when the solution will be used, the unused reagents are stored at 4℃.

Description

Succinate Dehydrogenase (SDH, EC 1.3.5.1) is widely found in animals, plants, microorganisms and cultured cells. SDH is a marker enzyme of mitochondria, which is a membrane binding enzyme located in the inner membrane of mitochondria. It is also one of the key points of respiratory electron transfer and oxidative phosphorylation. In addition, it provides electrons for the respiratory chain of various prokaryotic cells.

SDH can catalyze the dehydrogenation of succinic acid to fumaric acid. The dehydrogenation can reduce 2,6-dichlorophenol indophenol (DCPIP) under the transfer of phenazine dimethyl sulfate (PMS). 2,6-DCPIP has a characteristic absorption peak at 600 nm. The reduction rate of 2,6-DCPIP is determined by the change of absorbance at 600 nm, which represents the activity of SDH enzyme.

Required but not provided

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

Protocol

I. Extraction of SDH:

Accurately weigh 0.1 g of tissue or collect 5 million cells, add 1 mL of Reagent I and 10 μL of Reagent II, homogenize by using homogenizer/mortar in ice bath, fully grind, centrifuge at 11000×g for 10 minutes at 4℃, take the supernatant and place it on ice for test.

II. Procedure

1. Preheat spectrophotometer/microplate reader for 30 minutes, adjust wavelength to 600 nm, set zero with distilled water.

2. Procedure test

Reagent name (μL)	Test tube (T)	Black tube (B)
Reagent III	168	168
Reagent V	12	12
Keep warm at 25℃(general species) or 37℃(mammals) water bath for 10 minutes.		
Sample	10	-
Distilled water	-	10
Reagent VI	10	10

Add Reagent III and Reagent V to 1.5 mL EP tube in turn, after keep warm at 37℃ (mammal) or 25℃ (other species) for 10 minutes, add it into micro cuvette/96 well flat-bottom plate. Then add each reagent in turn according to the table, record the initial absorbance A1 at the wavelength of 600 nm for 20s and the absorbance A2 at the wavelength of 80s, ΔA = A1-A2, obtain ΔA_T, ΔA_B.

III. Calculation of SDH activity

A. Microplate reader

(1). Protein concentration

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the consume of 1 nmol of 2,6-dichlorophenol indophenol per minute in the reaction system every milligram tissue protein.

$SDH(U/mg\ prot)=[(\Delta A_T-\Delta A_B)\div(\varepsilon\times d)\times V_{RV}\times 10^9]\div(Cpr\times V_S)\div T=952.381\times(\Delta A_T-\Delta A_B)\div Cpr$

(2) Sample weight

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the consume of 1 nmol of 2,6-dichlorophenol indophenol per minute in the reaction system every gram tissue.

$SDH(U/g)=[(\Delta A_T-\Delta A_B)\div(\varepsilon\times d)\times V_{RV}\times 10^9]\div(V_S\div V_{ST}\times W)\div T=961.905\times(\Delta A_T-\Delta A_B)\div W$

(3) Germ or cells

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the consume of 1 nmol of 2,6-dichlorophenol indophenol per minute in the reaction system every 10 thousand germ or cells.

$SDH(U/10^4\ cell)=[(\Delta A_T-\Delta A_B)\div(\varepsilon\times d)\times V_{RV}\times 10^9]\div(V_S\div V_{ST}\times 500)\div T$

$1.924\times(\Delta A= \quad _T-\Delta A_B)$

V_{RT}: Total reaction volume, 2×10^{-4} L;

ε: The molar extinction coefficient of 2,6-DCPIP, 2.1×10^4 L/mol/cm;

d: The light diameter of cuvette, 1 cm;

Vs: Sample volume, 0.01 mL;

$V_{ST:}$ Add the volume of Reagent I and Reagent II, 1.01 mL;

T: Reaction time(min), 1 minute;

Cpr: Sample protein concentration, mg/mL;

W: Sample weight, g;

500: Cells or germ, 5 million.

b. 96 well plate：

(1) Protein concentration

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the consume of 1 nmol of 2,6-dichlorophenol indophenol per minute in the reaction system every milligram tissue protein.

$SDH(U/mg\ prot)=[(\Delta A_T-\Delta A_B)\div(\varepsilon\times d)\times V_{RV}\times 10^9]\div(Cpr\times V_S)\div T=1587.302\times(\Delta A_T-\Delta A_B)\div Cpr$

(2) Sample weight

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the consume of 1 nmol of 2,6-dichlorophenol indophenol per minute in the reaction system every gram tissue.

$SDH(U/g)=[(\Delta A_T-\Delta A_B)\div(\varepsilon\times d)\times V_{RV}\times 10^9]\div(V_S\div V_{ST}\times W)\div T=1603.175\times(\Delta A_T-\Delta A_B)\div W$

(3) Germ or cells

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the consume of 1 nmol of 2,6-dichlorophenol indophenol per minute in the reaction system every 10 thousand of germ or cells.

$SDH(U/10^4\ cell)=[(\Delta A_T-\Delta A_B)\div(\varepsilon\times d)\times V_{RV}\times 10^9]\div(V_S\div V_{ST}\times 500)\div T$

$3.206\times(\Delta A= {}_T-\Delta A_B)$

V_{RT}: Total reaction volume, 2×10^{-4} L;

ε: The molar extinction coefficient of 2,6-DCPIP, 2.1×10^4 L/mol/cm;

d: The light diameter of 96 well plate, 0.6 cm;

Vs: Sample volume, 0.01 mL;

$V_{ST:}$ Add the volume of Reagent I and Reagent II, 1.01 mL;

T: Reaction time, 1 minute;

Cpr: Sample protein concentration, mg/mL;

W: Sample weight, g;

500: Cells or germ, 5 million.

Note

1. All reagents and samples shall be placed on ice during the determination to avoid denaturation and deactivation.
2. If ΔA is greater than 0.5(cuvette)/0.3(96 well flat-bottom plate), the enzyme solution should be diluted with enzyme extract to obtain ΔA with less than 0.5/0.3, which can improve the detection sensitivity.
3. Because the Extract solution contains a certain concentration of protein (about 1 mg/mL), it is necessary to subtract the protein content of the Extract solution itself when determining the protein concentration of the sample.

Experimental example:

1. Take 0.1g of kidney, add 1 mL of Reagent I and 10 μL Reagent II, grind the homogenate with ice bath, centrifuge at 4℃ and 11000g for 10min, and place the supernatant on ice. According to the determination procedure, the enzyme activity is calculated as follows: $\Delta A_T= A1_T- A2_T= 0.7838-0.6414 = 0.1424$, $\Delta A_B= A1_B- A2_B= 1.019-1.019 = 0$

SDH activity (U/g mass) = 961.905×(ΔA_T- ΔA_B) ÷ W = 1369.75 U/g mass.

References：

[1] Fattoretti P, Bertoni-Freddari C, Caselli U, et al. Impaired succinic dehydrogenase activity of rat Purkinje cell mitochondria during aging[J]. Mechanisms of ageing and development, 1998, 101(1-2): 175-182.

Related Products：

NA0812/NA0570 α-Ketoglutarate Dehydrogenase(α-KGDH) Activity Assay Kit

NA0717/NA0476 Citric Acid(CA) Content Assay Kit

NA0837/NA0595 Pyruvate Dehydrogenase(PDH) Activity Assay Kit