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Succinate Dehydrogenase (SDH) Activity Assay Kit

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

Detection equipment: Spectrophotometer

Catalog Number: AK0504-50T-48S

Product Composition: Before use, please carefully check whether the volume of the reagent is consistent with the volume in the bottle. If you have any questions, please contact salesman in time.

Reagent name	Size	Preservation Condition
Reagent I	Liquid 60 mL×1	-20℃
Reagent II	Liquid 0.6 mL×1	-20℃
Reagent III	Liquid 50 mL×1	2-8℃
Reagent IV	Liquid 3mL×1	2-8℃
Reagent V	Liquid 3mL×1	2-8℃

Solution Preparation:

Reagent II: Volatile reagent, sealed as soon as possible after use, storage at -20℃.

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 catalyzes 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, water-bath, tabletop centrifuge, adjustable pipette, 1 mL glass cuvette, mortar/homogenizer/cell ultrasonic crusher, ice and distilled water.

Operation procedure:

I. Sample preparation (The sample size to be tested can be adjusted appropriately, and the specific proportion can be referred to the literature.)

1. Tissue sample: weigh about 0.1g of tissue, add 1mL of reagent I and 10μL of reagent II, grind thoroughly with an ice bath homogenizer or mortar, centrifuge at 11000g for 10min at 4℃, remove supernatant and place on ice for measurement.

2. Cell or bacterial samples: first collect 5 million bacteria/cell into a centrifuge tube, centrifuge and discard the supernatant; then add 1 mL of reagent I and 10 μL of reagent II, ultrasonicate the bacteria in ice bath (power 200W, 3s, 7s interval, total time 5min); then centrifuge for 10 min at 11000g, 4℃. The supernatant was placed on ice for testing.

II. Determination procedure

1. Preheat spectrophotometer for more than 30 minutes, adjust wavelength to 600 nm and set zero with distilled water.
2. Before use, preheat reagent III at 37℃ (mammals) or 25℃ (other species) for 10min according to the sample size.
3. Operation table

Reagent name (μL)	Test tube (T)	Black tube (B)
Reagent III	850	850
Reagent IV	50	50
Sample	50	-
Distilled water	-	50
Reagent V	50	50
After full mixing, the initial absorbance A1 at the 600nm wavelength of 20s was immediately determined. Then it is quickly placed at 37℃ (mammals) or 25℃ (other species) for 5min, and the absorbance A2 at 5min20s is measured. Calculate ΔA=A1-A2 to to get ΔA_T, ΔA_B. The blank tube only needs to be tested 1-2 times.		

III. Calculation of SDH activity

Calculation formula for determination with 1 mL glass cuvette.

(1) Calculate by sample protein concentration

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

$SDH(U/mg\ prot)=[(\Delta A_T-\Delta A_B)\div(\varepsilon\times d)\times V_{RV}\times 10^9]\div(Cpr\times V_S)\div T=190.476\times(\Delta A_T-\Delta A_B)\div Cpr$

(2) Calculate by sample mass

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

$SDH(U/g\ mass)=[(\Delta A_T-\Delta A_B)\div(\varepsilon\times d)\times V_{RV}\times 10^9]\div(V_S\div V_{ST}\times W)\div T=192.381\times(\Delta A_T-\Delta A_B)\div W$

(3) Calculate by the number of bacteria or cells

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

$SDH(U/10^4\ cell)=[(\Delta A_T-\Delta A_B)\div(\varepsilon\times d)\times V_{RV}\times 10^9]\div(V_S\div V_{ST}\times N)\div T=192.381\times(\Delta A_T-\Delta A_B)\div N$

V_{RT}: Total reaction volume, 1×10^{-3} 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, 0.05 mL;

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

T: Reaction time(min), 5 minute;

Cpr: Sample protein concentration, mg/mL;

W: Sample mass, g;

N: Cells or bacteria, million;

10^9：1mol=10^9nmol.

Note:

1. All reagents (Not preheating reagent) and samples shall be placed on ice during the determination to avoid denaturation and deactivation.
2. If ΔA is greater than 0.6, the enzyme solution should be diluted with distilled water to obtain ΔA with less than 0.6, which can improve the detection sensitivity. Change the calculation formula simultaneously.
3. The Reagent I contains a certain concentration of protein (about 1 mg/mL), it is necessary to subtract the protein content of the Reagent I itself when determining the protein concentration of the sample.

Experimental example:

1. Take 0.1g of Rat 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: $\Delta A_T = A1_T - A2_T$ =1.071-0.845=0.226, $\Delta A_B = A1_B - A2_B$ =1.180-1.180=0

   SDH activity (U/g mass) =192.381×(ΔA_T- ΔA_B) ÷ W =418.059 U/g mass.