Succinate Dehydrogenase (SDH)Activity Assay Kit

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

Detection equipment: Spectrophotometer/Microplate reader

Cat No: BC0955

Size: 100T/96S

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 Solarbio staff in time.

Reagent name	Size	Preservation Condition
Reagent I	Liquid 110 mL×1	-20℃
Reagent II	Liquid 0.6 mL×2	-20℃
Reagent III	Liquid 18 mL×1	2-8℃
Reagent IV	Liquid 1.5mL×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/Microplate reader, water-bath, desk centrifuge, adjustable pipette, micro cuvette/96 well flat-bottom plate, mortar/homogenizer, 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/microplate reader for 30 minutes, adjust wavelength to 600 nm, 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(added to 96 well plate / micro glass cuvette)

Reagent name (μL)	Test tube (T)	Black tube (B)
Reagent III	170	170
Reagent IV	10	10
Sample	10	-
Distilled water	-	10
Reagent V	10	10
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

A. Microplate reader

(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.

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

(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, 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, 5 minute;

Cpr: Sample protein concentration, mg/mL;

W: Sample weight, g;

N: Cells or bacteria, million;

10^9:1mol=10^9nmol.

b. 96 well plate:

(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.

$SDH(U/mg\ prot)=[(\Delta A_T-\Delta A_B)\div(\varepsilon\times d)\times V_{RV}\times 10^9]\div(Cpr\times V_S)\div T=317.460\times(\Delta A_T-\Delta A_B)\div Cpr$

(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.

$SDH(U/g)=[(\Delta A_T-\Delta A_B)\div(\varepsilon\times d)\times V_{RV}\times 10^9]\div(V_S\div V_{ST}\times W)\div T=320.635\times(\Delta A_T-\Delta A_B)\div W$

(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 of bacteria or cells.

$SDH(U/10^4\ cell)=[(\Delta A_T-\Delta A_B)\div(\varepsilon\times d)\times V_{RV}\times 10^9]\div(V_S\div V_{ST}\times N)\div N=320.635\times(\Delta A_T-\Delta A_B)\div N$

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, 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(cuvette)/0.4(96 well flat-bottom plate), the enzyme solution should be diluted with distilled water to obtain ΔA with less than 0.5/0.3, 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 liver, 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 by 96 well plate as follows:
   $\Delta A_T = A1_T - A2_T = 0.561-0.208=0.353$, $\Delta A_B = A1_B - A2_B = 0.814-0.814=0$
   SDH activity (U/g mass) $=320.635\times(\Delta A_T - \Delta A_B) \div W = 1091.3458$ U/g mass.

Related products:

[1] Chong D, Gu Y, Zhang T, Xu Y, Bu D, Chen Z, Xu N, Li L, Zhu X, Wang H, Li Y, Zheng F, Wang D, Li P, Xu L, Hu Z, Li C. Neonatal ketone body elevation regulates postnatal heart development by promoting cardiomyocyte mitochondrial maturation and metabolic reprogramming. Cell Discov. 2022 Oct 11;8(1):106. doi: 10.1038/s41421-022-00447-6. PMID: 36220812; PMCID: PMC9553951.

[2] Cai B, Ma M, Zhang J, Wang Z, Kong S, Zhou Z, Lian L, Zhang J, Li J, Wang Y, Li H, Zhang X, Nie Q. LncEDCH1 improves mitochondrial function to reduce muscle atrophy by interacting with SERCA2. Mol Ther Nucleic Acids. 2021 Dec 10;27:319-334. doi: 10.1016/j.omtn.2021.12.004. PMID: 35024244; PMCID: PMC8717430.

[3] Ma M, Cai B, Zhou Z, Kong S, Zhang J, Xu H, Zhang X, Nie Q. LncRNA-TBP mediates TATA-binding protein recruitment to regulate myogenesis and induce slow-twitch myofibers. Cell Commun Signal. 2023 Jan 12;21(1):7. doi: 10.1186/s12964-022-01001-3. PMID: 36635672; PMCID: PMC9835232.

[4] Zhang Z, Luo W, Chen G, Chen J, Lin S, Ren T, Lin Z, Zhao C, Wen H, Nie Q, Meng X, Zhang X. Chicken muscle antibody array reveals the regulations of LDHA on myoblast differentiation through energy metabolism. Int J Biol Macromol. 2024 Jan;254(Pt 1):127629. doi: 10.1016/j.ijbiomac.2023.127629. Epub 2023 Oct 25. PMID: 37890747.

[5] Wang Q, Zhou X, Gou H, Chang H, Lan J, Li J, Li Z, Gao M, Wang Z, Yi Y, Li N. Antibacterial activity of a polysaccharide isolated from Artemisia argyi leaf against Staphylococcus aureus and mechanism investigation. Int J Biol Macromol. 2023 Dec 31;253(Pt 1):126636. doi: 10.1016/j.ijbiomac.2023.126636. Epub 2023 Aug 30. PMID: 37657565.

References:

[1] Cimen H, Han MJ, Yang Y. et al. Regulation of succinate dehydrogenase activity by SIRT3 in mammalian mitochondria[J]. Biochemistry, 2010, 49(2): 304-311.

Related Products:

BC0710/BC0715	α-Ketoglutarate Dehydrogenase (α-KGDH) Activity Assay Kit
BC2150/BC2155	Citric Acid (CA) Content Assay Kit
BC0380/BC0385	Pyruvate Dehydrogenase (PDH) Activity Assay Kit