(FOR RESEARCH USE ONLY. DO NOT USE IT IN CLINICAL DIAGNOSIS!)

Succinate Dehydrogenase (SDH) Assay Kit

Catalog No.: BC00175

Size: 50T

Please read the instructions carefully before use. If you have any questions or need further help during experiment, please don't hesitate to contact us through the following methods:

✉ Email (Sale) order@enkilife.com

✉ Email (Techsupport) techsupport@enkilife.com

☎ Tel: 0086-27-87002838

Website: www.enkilife.com

Shelf life: Please refer to the label on the outer package.

Techsupport: In order to provide you with better service, please inform us the lot number on the label of the outer package.

Basic Information

Product Name	Succinate Dehydrogenase (SDH) Assay Kit
Detection Method	Colorimetric
Sample Type	Tissue, serum, plasma
Assay Type	Quantitative
Detection Instrument	Spectrophotometer (600 nm)

Product Introduction

Succinate dehydrogenase is a membrane-bound enzyme located on the inner mitochondrial membrane. Serving as one of the hubs linking oxidative phosphorylation and electron transport, it donates electrons to the aerobic energy-producing respiratory chains in eukaryotic mitochondria and various prokaryotic cells, and it is a marker enzyme of mitochondria.

As a key enzyme involved in the tricarboxylic acid (TCA) cycle, succinate dehydrogenase acts as an indicator of mitochondrial function. Its activity is commonly used to assess the operational efficiency of the TCA cycle. It is of great significance for evaluating sperm mitochondrial function and investigating the pathological mechanisms underlying ketosis in dairy cows.

Principle

Succinate dehydrogenase (SDH) catalyzes substrate reactions with FAD as the prosthetic group of this reaction. FAD is reduced to $FADH_2$, and this reaction is coupled to the reduction of 2,6-DPIP. The activity of SDH can be calculated by measuring the reduction rate of 2,6-DPIP.

Components

No.	Components	Size	Notes
Reagent 1	Liquid	60 mL×2	Store at 4°C
Reagent 2	Liquid	6 mL	Store at 4°C, protected from light
Reagent 3	Liquid	6 mL	Store at 4°C, protected from light
Reagent 4	Liquid	6 mL×2	Store at 4°C, protected from light

Reagent 5	Liquid	6 mL	Store at -20 °C protected from light. If repeated use is required, aliquot and freeze the solution to avoid repeated freeze-thaw cycles.
Reagent 6	Liquid	6 mL	Store at 4°C

Preparation of Working Solution: Mix Reagent 1, Reagent 2, Reagent 3, Reagent 4, Reagent 5 and Reagent 6 at a volume ratio of 2: 0.1: 0.1: 0.2: 0.1: 0.1. Prepare only the amount required for immediate use. Fresh working solution must be prepared right before testing and protected from light after preparation.

Storage

The kit has a validity period of 3 months.

Preparation

Visible light spectrophotometer with 1 cm path-length cuvettes, 37°C water bath, vortex mixer, stopwatch, protein quantification reagents (for tissue samples, available from our company).

- **Sample handling**

1. Tissue Samples: Accurately weigh the tissue sample, add 9 volumes of normal saline at a tissue weight (g) to liquid volume (mL) ratio of 1:9. Mechanically homogenize the mixture in an ice water bath, then centrifuge at 2500 rpm for 10 minutes. Collect the supernatant for subsequent measurement. A portion of the supernatant is simultaneously taken to determine the protein concentration.

2. Serum/Plasma: Take whole blood with or without anticoagulant, centrifuge at 1000-1500 rpm for 8 minutes, and collect the upper serum or plasma layer for testing.

Operation process

1. Set the visible spectrophotometer to 600 nm with 1 cm path-length cuvettes, and zero the instrument using double-distilled water. Prepare two cuvettes: one for zero adjustment and the other for sample measurement.
2. Pre-warm the working solution at 37 °C in the dark for no less than 5 minutes.

3. Add 100 μL test sample into a test tube, rapidly pipette 2.6 mL working solution into the tube, mix thoroughly immediately and start timing.
4. Transfer the mixture quickly into the cuvette, measure absorbance at 600 nm on the spectrophotometer. Record the absorbance value (A_1) at the 10-second mark, then read the absorbance value (A_2) again at 1 minute and 10 seconds.
5. Calculate the absorbance difference between the two readings: $\Delta A = A_1 - A_2$.

Calculation

1. Serum (plasma) SDH activity:

Definition: One specific activity unit is defined as a 0.01 decrease in the absorbance of the reaction system per milliliter of serum per minute.

Calculation formula:

$$\text{SDH activity (U/mL)} = \Delta A \div 0.01 \div T \div V_{Sample}$$

2. NOX activity in tissues or cells:

Definition: One specific activity unit is defined as a 0.01 reduction in absorbance of the reaction system per milligram of protein per minute.

Calculation formula:

$$\text{SDH activity (U/mg)} = \Delta A \div 0.01 \div T \div (V_{Sample} \times Cpr)$$

Annotation:

V_{Sample}: Volume of sample added, 0.1 mL

T: Reaction time, 1 min

Cpr: Sample protein concentration, mg/mL

Precautions

1. It is recommended to use disposable laboratory test tubes for operation.
2. This product is intended for scientific research use only by professionals and must not be used for clinical diagnosis or treatment, in food or drugs, or stored in ordinary residences.

3. The working solution must be stored away from light, and light protection is also required during measurement.
4. Cuvettes shall be thoroughly rinsed with tap water, followed by 2-3 washes with double-distilled water before testing the next sample.
5. After loading the sample into the cuvette, reagent addition and stopwatch timing must be performed simultaneously.
6. Immediately transfer the cuvette to the spectrophotometer cell holder right after reagent loading.
7. Reagent 2, Reagent 3, Reagent 4 and Reagent 5 shall be refrigerated and protected from light.
8. The prepared mixed working solution must be pre-warmed prior to measurement.