# Colorimetric Succinate Dehydrogenase Activity Assay Kit

## Introduction

Succinate dehydrogenase (SDH) is a cytochrome oxidase located on the inner mitochondrial membrane that provides electrons for the respiratory chain. SDH catalyzes the oxidation of succinic acid to fumarate while transferring electrons to coenzyme Q, which provides energy to the cell through the tricarboxylic acid cycle and electron transport chain. Therefore, SDH activity can be used as an indicator to evaluate the tricarboxylic acid cycle. In addition, SDH mutations may lead to many diseases, such as Ritchie syndrome. Detection of SDH activity is of great clinical significance.

The Colorimetric Succinate Dehydrogenase Activity Assay Kit is used for the rapid detection of SDH activity by colorimetric method. SDH dehydrogenates succinic acid to produce fumarate, and the removed hydrogen is delivered via PMS and reduces DCIP. DCIP is a blue substance with an absorption peak at 600 nm. The degree of reduction of DCIP can be judged by detecting changes in absorbance, which indirectly reflects the activity of SDH. SDH activity is inversely proportional to the amount of residual DCIP.

This product is rapid, simple and highly sensitive. It has a good linearity in the range of 0-100 nmol.

## Components and Storage

| Components \ Size | 100 Assays | Storage |
|---|---|---|
| **Lysis Buffer** | 25 mL | -20°C |
| **SDH Assay Buffer** | 20 mL | -20°C |
| **DCIP (50 mM)** | 200 μL | -20°C away from light |
| **SDH Substrate** | 120 μL | -20°C |
| **SDH Probe** | 100 μL | -20°C away from light |
| **Shipping: Blue ice** | **Shelf life: 1 year** | |

## Protocol

### 1. Sample preparation

1) Serum samples: Keep whole blood at room temperature for 0.5-2 h without vigorous shaking to avoid hemolysis. After the whole blood naturally coagulates, centrifuge at 4°C, 1000-2000 g for 10 min. Take the yellow supernatant as serum. Transfer the serum to ice for later use, and if testing is not immediately

available, aliquot and store briefly at -20°C or -80°C. Thaw and mix cryopreserved samples before use.

2) Plasma sample: Anticoagulated whole blood with heparin or EDTA, centrifuged at 4°C, 1000-2000 g for 10 min. Take the yellow supernatant as plasma. Transfer plasma to ice for later use, and if not immediately detected, aliquot and store briefly at -20°C or -80°C. Thaw and mix cryopreserved samples before use.

3) Cell samples: For adherent cells, wash the cells once with PBS. For suspension cells, collect the cells by centrifugation. Add 100-200 μL of Lysis Buffer per $1\times10^6$ cells, pipette appropriately, then incubate on ice for 5-10 mins to fully lyse the cells. Centrifuge at 4°C, 12,000 g for 3-5 min, and transfer the supernatant on ice for later use. If not used immediately, aliquot and store at -20°C or -80°C for a short time.

4) Cell culture supernatant: Take the supernatant directly for later use.

5) Tissue samples: Add 100 μL of Lysis Buffer per 10 mg sample to homogenize on ice. Centrifuge at 4°C, 12,000 g for 3-5 min, and transfer the supernatant on ice for later use. If not used immediately, aliquot and store at -20°C or -80°C for a short time.

6) Mitochondrial samples: Mitochondria from fresh tissues and cells are recommended.

***Note:** Mitochondria can be extracted using the Cell Mitochondria Isolation Kit I (K2724) or the Tissue Mitochondria Isolation Kit (K2725).

## 2. SDH working solution preparation

1) Warm Lysis Buffer and SDH Assay Buffer to room temperature in advance. Place DCIP (50 mM) in a 37°C water bath for 5 min. Keep other reagents on ice. Put them back in place immediately after use.

2) Refer to the following table to prepare the SDH detection working solution, and the working solution is best prepared and used now. The prepared working solution can be stored at 4°C or on ice in the dark, and used on the same day.

| Sample number | 1 | 10 | 100 |
|---|---|---|---|
| SDH Assay Buffer | 47 μL | 470 μL | 4700 μL |
| DCIP (50 mM) | 1 μL | 10 μL | 100 μL |
| SDH Substrate | 1 μL | 10 μL | 100 μL |
| SDH Probe | 1 μL | 10 μL | 100 μL |
| Total | 50 μL | 500 μL | 5000 μL |

***Note:**

a) Because DCIP (50 mM) and SDH Probe are used in small amounts and tend to settle, it is recommended to vortex and centrifuge before use.

b) If there are substances in the sample that react with PMS, it will interfere with the detection of SDH activity. At this time, it is necessary to set up a background control, and use SDH Assay Buffer instead of SDH Substrate for the preparation of SDH working solution.

3. **DCIP standard:** Add 8 μL of DCIP (50 mM) to 192 μL of SDH Assay Buffer and mix to make DCIP (2 mM). Transfer 0, 0.5, 1, 2.5, 5, 10, 20, 30, 40, and 50 μL of DCIP (2 mM) to a 96-well plate, adjust the volume to 50 μL with SDH Assay Buffer. At this time, the final standard concentrations are 0, 1, 2, 5, 10, 20, 40, 60, 80, 100 nmol.

4. **SDH activity assay**

   1) Add 1-50 μL of sample or diluted sample to the 96-well plate, supplement to 50 μL with SDH Assay Buffer. At the same time, set up a blank control containing only SDH Assay Buffer.

***Note:** To ensure that the sample value falls within the standard curve, it is recommended to make multiple dilutions of the sample, at which point the dilution factor can be denoted as n. If a 10-fold dilution is applied to the sample, 10 μL of the diluted sample is used, then n = 10×50/10 = 50.

   2) Add 50 μL of SDH Assay Solution to each sample well, and add 50 μL of SDH Assay Buffer to each standard well, and mix well.

   3) Immediately determine the absorbance at 600 nm in kinetic mode, and it is recommended to record for 10-30 min. select two time-points ($T_1$ and $T_2$) and record the signal as $A_1$ and $A_2$.the change in signal ($\Delta A=A_1 - A_2$) reflects the amount of DCIP.

5. **Data analysis**

   1) Subtract the signal of the blank control from both the sample and the standard value.

   2) Establish a standard curve and bring ΔA into the standard curve to calculate the amount of DCIP of the sample (C) during the reaction time.

***Note:** If the background control group has a high value, subtract the background control reading from the sample reading.

   3) SDH activity unit definition: one unit (U) is the amount of enzyme that catalyzes 1 μmol DCIP per 1 min at 25°C, pH 7.2.

$$\text{SDH Activity (nmol/min/μL or mU/μL or U/mL)} = C \times n/(\Delta T \times V)$$

Note: C is the amount of DCIP (nmol) in the sample calculated from the standard curve; ΔT is the reaction time (min); V is the sample volume (50 μL)

## Note

1. DCIP (50 mM) may precipitate after freezing and thawing, so put it in a 37°C water bath for 5 min and mix well before use.

2. For your safety and health, please wear a lab coat and disposable gloves for operation.

3. This product is for scientific use only and should not be used for clinical diagnosis or treatment.

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