Succinate Dehydrogenase Microplate Assay Kit

Basic information:

Catalog No.: UAK1070 **Size:** 100 Assays

For research use only. Not for diagnostic or therapeutic procedures.

I. INTRODUCTION

Succinate Dehydrogenase (SDH) (EC 1.3.5.1) or succinate-coenzyme Q reductase (SQR) or respiratory complex II is an enzyme complex, which is bound to the inner mitochondrial membrane. SDH participates in both the citric acid cycle and electron transport chain. In mammals and many bacteria, SDH consists of 2 hydrophilic subunits, SDHA (flavoprotein) and SDHB (iron-sulfur protein) and 2 hydrophobic membrane anchor subunits: SDHC and SDHD. SDH oxidizes succinate to fumarate and transfers the electrons to ubiquinone. SDH deficiency in humans leads to a variety of phenotypes including Leigh syndrome, a neurometabolic disorder, tumor formation, and myopathy. Recent studies show that SDH can prevent the generation of ROS (reactive oxygen species); therefore, measurement of succinate dehydrogenase activity has wide applications.

The assay is initiated with the enzymatic hydrolysis of Succinic acid by SDH. The enzyme catalysed reaction products can be measured at a colorimetric readout at 600 nm.

II. KIT COMPONENTS

Component	Volume	Storage
96-Well Microplate	1 plate	
Assay Buffer I	30 ml x 4	4 °C
Assay Buffer II	1.2 ml x 1	4 °C
Assay Buffer III	20 ml x 1	4 °C
Substrate	Powder x 1	4 °C
Probe	Powder x 1	-20 °C

Technical Manual	1 Manual	

Note:

Substrate*: add 18 ml distilled water to dissolve before use.*

Probe*: add 1 ml distilled water to dissolve before use.*

III. MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 600 nm

2. Distilled water

3. Pipettor

4. Pipette tips

5. Mortar

6. Centrifuge

7. Timer

8. Ice

IV. SAMPLE PREPARATION

1. For cell and bacteria samples

Collect cell or bacteria into centrifuge tube, discard the supernatant after centrifugation, add 0.99 ml Assay Buffer I and 10 µl Assay Buffer II on ice, centrifuged at 600g 4 °C for 5 minutes. Take the supernatant into a new centrifuge tube, 11000g 4 °C for 10 minutes, discard the supernatant. Add 198 µl Assay Buffer III and 2 µl Assay Buffer II to the precipitation, shock, sonicate (with power 20%, sonication 3s, intervation 10s, repeat 30 times). Centrifuged at 11000g 4 °C for 10 minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

2. For tissue samples

Weigh out 0.1 g tissue, homogenize with 0.99 ml Assay Buffer I and 10 µl Assay Buffer II on ice, centrifuged at 600g 4 °C for 5 minutes. Take the supernatant into a new centrifuge tube, 11000g 4 °C for 10 minutes, discard the supernatant. Add 198 µl Assay Buffer III and 2 µl Assay Buffer II to the precipitation, shock, sonicate (with power 20%, sonication 3s, intervation 10s, repeat 30 times). Centrifuged at 11000g 4 °C for 10 minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

V. ASSAY PROCEDURE

Add following reagents into the microplate:

Reagent	Sample
Sample	10 μl
Probe	10 μl
Substrate	180 μl
Mix, measured at 600 nm and record the absorbance of 20th second and 80th second.	

VI. CALCULATION

Unit Definition: One unit of SDH activity is defined as the enzyme reduces 1 nmol of DCPIP per minute.

1. According to the protein concentration of sample

$$SDH\ (U/mg) = (OD_{Sample(20S)} - OD_{Sample(80S)}) / (\varepsilon \times d) \times V_{Total} \times 10^9 / (V_{Sample} \times C_{Protein}) / T$$

$$= 1587 \times (OD_{Sample(20S)} - OD_{Sample(80S)}) / C_{Protein}$$

2. According to the weight of sample

$$SDH\ (U/g) = (OD_{Sample(20S)} - OD_{Sample(80S)}) / (\varepsilon \times d) \times V_{Total} \times 10^9 / (W \times V_{Sample} / V_{Assay}) / T$$

$$= 317 \times (OD_{Sample(20S)} - OD_{Sample(80S)}) / W$$

3. According to the quantity of cells or bacteria

$$SDH\ (U/10^4) = (OD_{Sample(20S)} - OD_{Sample(80S)}) / (\varepsilon \times d) \times V_{Total} \times 10^9 / (N \times V_{Sample} / V_{Assay}) / T$$

$$= 317 \times (OD_{Sample(20S)} - OD_{Sample(80S)}) / N$$

ε: molar extinction coefficient, 21×10^3 L/mol/cm;

d: the optical path of 96-Well microplate, 0.6 cm;

$C_{Protein}$: the protein concentration, mg/ml;

W: the weight of sample, g;

N: the quantity of cell or bacteria, $N \times 10^4$;

V_{Total}: the total volume of the enzymatic reaction, 0.2 ml;

V_{Sample}: the volume of sample, 0.01 ml;

V_{Assay}: the volume of Assay buffer in sample preparation, 0.2 ml;

T: the reaction time, 1 minute.