



Succinate Dehydrogenase (Mitochondrial Complex II) Activity Colorimetric Microplate Assay Kit User Manual

Catalog # CAK1070

(Version 1.7E)

Detection and Quantification of Succinate Dehydrogenase
(Mitochondrial Complex II) Activity in Urine, Serum, Plasma, Tissue
extracts, Cell lysate, Cell culture media and Other biological
fluids Samples.

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

I. INTRODUCTION.....	2
II. KIT COMPONENTS.....	3
III. MATERIALS REQUIRED BUT NOT PROVIDED.....	4
IV. REAGENT PREPARATION.....	5
V. SAMPLE PREPARATION.....	6
VI. ASSAY PROCEDURE.....	7
VII. CALCULATION.....	8
VIII.TYPICAL DATA.....	9

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.

Succinate Dehydrogenase Activity Colorimetric Microplate Assay Kit provides a simple and direct procedure for measuring succinate dehydrogenase activity levels in a variety of samples. 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 450 nm.

II.KIT COMPONENTS

Component	Volume	Storage
96-Well Microplate	1 plate	
Assay Buffer I	30 mlx 4	4 °C
Assay Buffer II	1.2 mlx 1	4 °C
Assay Buffer III	20 mlx 1	4 °C
Substrate Dilution	10 mlx 1	4 °C
Substrate	Powderx 1	4 °C
Dye ReagentA	Powder x 1	4 °C
Dye Reagent B	1 ml x 1	4 °C
Standard	Powder x 1	-20 °C
Technical Manual	1 Manual	

III. MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 450 nm
2. Distilled water
3. Pipettor, multi-channel pipettor
4. Pipette tips
5. Mortar
6. Centrifuge
7. Timer
8. Ice

IV. REAGENT PREPARATION

Standard: Briefly centrifuge prior to opening. Dissolve in 1 ml distilled water to generate 2 mmol/L of standard stock solution, store at 4 °C for 1 day or -20°C for 3 days after reconstitution. Then dilute to 600 µmol/L standard top solution by adding 0.3 ml stock solution into 0.7 ml distilled water. Perform 2-fold serial dilutions of the top standard solution using distilled water to make the standard curve. The concentration of standard curve could be 600/300/150/75/37.5/18.75 µmol/L.

Substrate: Add 9 ml Substrate Dilution to dissolve before use. Store at 4 °C for 1-2 weeks or -20°C for 2-3 months after reconstitution.

Dye Reagent A: Add 9 ml distilled water to dissolve before use. Keep in dark and store at 4°C for 1 week or -20°C for 1 month.

Note: Divide into small aliquots to avoid repeated freeze-thaw cycles.

V. 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 μ l Assay Buffer II on ice, 10000g 4°C for 10 minutes, discard the supernatant. Add 198 μ l Assay Buffer III and 2 μ l Assay Buffer II to the precipitation, shock, sonicate (with power 20%, sonicate 3s, interval 10s, repeat 30 times). Then add 800 μ l distilled water, mix, centrifuged at 10000g 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 μ l Assay Buffer II on ice, 10000g 4°C for 10 minutes, discard the supernatant. Add 198 μ l Assay Buffer III and 2 μ l Assay Buffer II to the precipitation, shock, sonicate (with power 20%, sonicate 3s, interval 10s, repeat 30 times). Then add 800 μ l distilled water, mix, centrifuged at 10000g 4°C for 10 minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

VI. ASSAY PROCEDURE

Warm all reagents to room temperature before use.

Add following reagents into the microplate:

Reagent*	Sample**	Blank	Standard
Sample	10 μ l	--	--
Substrate	90 μ l	--	--
Standard	--	--	100 μ l
Distilled water	--	100 μ l	--
Dye Reagent A	90 μ l	90 μ l	90 μ l
Dye Reagent B	10 μ l	10 μ l	10 μ l
Mix, incubate at 37 °C for 2 minutes, measured at 450 nm and record the absorbance.			

Note:

*Reagents must be added sequentially and should not be premixed prior to addition.

**For unknown samples, we recommend doing a pilot experiment & testing several doses to ensure the readings are within the standard curve range. If the enzyme activity is lower, please add more samples into the reaction system; or increase the reaction time; if the enzyme activity is higher, please dilute the sample, or decrease the reaction time.

**For colored samples, we recommend setting a parallel sample background control well with same volume of sample only. Other reagents were replaced by distilled water to the same total volume. Subtract the OD value of the sample background control from the OD value of the sample to correct for interference from the sample's own color.

VII. CALCULATION

Unit Definition: One unit of SDH activity is defined as the enzyme produce 1 μmol NADH per minute.

1. According to the slope of the standard curve

$$\text{Activity} = \frac{(\text{OD}_{\text{Sample}} - \text{OD}_{\text{Blank}}) - \text{Intercept}}{\text{Slope} \times T} \times \frac{V_{\text{Standard}}}{V_{\text{Sample}}} \times n (\text{U/mL})$$

2. According to one point of the standard OD value and concentration

2.1 According to the protein concentration of sample

$$\text{Activity} = \frac{(C_{\text{Standard}} \times V_{\text{Standard}}) \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Blank}})}{(\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) \times V_{\text{Sample}} \times C_{\text{Protein}} \times T} (\text{U/mg})$$

2.2 According to the quantity of cells or bacteria

$$\text{Activity} = \frac{(C_{\text{Standard}} \times V_{\text{Standard}}) \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Blank}})}{(\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) \times N \times (V_{\text{Sample}} / V_{\text{Assay}}) \times T} (\text{U}/10^4)$$

2.3 According to the weight of sample

$$\text{Activity} = \frac{(C_{\text{Standard}} \times V_{\text{Standard}}) \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Blank}})}{(\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) \times W \times (V_{\text{Sample}} / V_{\text{Assay}}) \times T} (\text{U/g})$$

2.4 According to the volume of sample

$$\text{Activity} = \frac{(C_{\text{Standard}} \times V_{\text{Standard}}) \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Blank}})}{(\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) \times V_{\text{Sample}} \times T} (\text{U/mL})$$

Slope: the absorbance slope of standard curve

n: the dilution factor

C_{Protein}: the protein concentration of sample, mg/mL

W: the weight of total sample, g

N: the quantity of total cell or bacteria sample, 10⁴

C_{Standard}: the concentration of standard, μmol/L

V_{Standard}: the volume of standard in assay procedure, mL

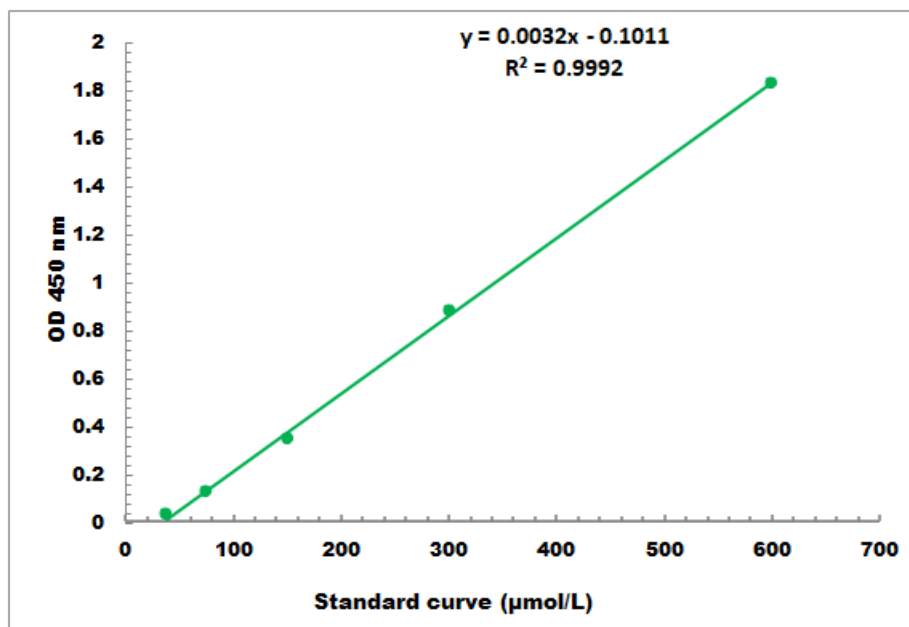
V_{Sample}: the volume of sample in assay procedure, mL

V_{Assay} : the volume of Assay Buffer II, Assay Buffer III and distilled water, in sample preparation, mL

T: the reaction time, minute

VIII. TYPICAL DATA

The standard curve is for demonstration only. A standard curve must be run with each assay.



Detection Range: 30 µmol/L -600 µmol/L