Succinate Dehydrogenase (SDH) Assay Kit

Catalog No.: abx298836

Size: 100 Assays

Storage: Store the standard at -20°C all other kit components in the dark at 4°C.

Application: For quantitative detection of Succinate Dehydrogenase activity in serum, plasma and other biological fluids.

Detection Range: 30 nmol/ml - 600 nmol/ml

Introduction

Succinate Dehydrogenase (SDH) is a membrane-bound metabolic enzyme that plays a critical role in the citric acid cycle and electron transport chain in eukaryotes and prokaryotes. SDH catalyzes redox reactions, reducing ubiquinone by the oxidization of succinate to fumarate. SDH deficiency in humans leads to a variety of phenotypes including Leigh syndrome, a neurometabolic disorder, tumor formation, and myopathy. Recent studies show that the oxidizing ability of SDH can prevent the generation of ROS (reactive oxygen species), a type of free radical species implicated in DNA, RNA and protein damage.

Abbexa's Succinate Dehydrogenase Assay Kit is a quick, convenient, and sensitive method for measuring and calculating SDH activity. The absorbance should be measured at 450 nm. The intensity of the color is proportional to the activity of the SDH enzyme, which can then be calculated.

Kit components

1. 96 well microplate
2. Assay Buffer I: 4 × 30 ml
3. Assay Buffer II: 1.2 ml
4. Assay Buffer III: 20 ml
5. Diluent buffer: 10 ml
6. Substrate: 1 vial
7. Dye Reagent A: 1 vial
8. Dye Reagent B: 1 ml
9. Standard: 1 vial

Materials Required But Not Provided

1. Microplate reader (450 nm)
2. Microcentrifuge tubes
3. High-precision pipette and sterile pipette tips
4. Distilled water
5. Mortar
6. Ice
7. Centrifuge and centrifuge tubes
8. Timer

For Reference Only

Protocol

A. Preparation of Sample and Reagents

1. Reagents

- **Substrate Working Solution**
  Add 9ml of Diluent Buffer into the Substrate vial and mix thoroughly to prepare the Substrate working solution. Ensure that the Substrate has completely dissolved prior to use.

- **Dye Reagent A Working Solution**
  Add 9 ml of distilled water into the Dye Reagent A vial and mix thoroughly to prepare the Dye Reagent Solution. Ensure that the Dye Reagent has completely dissolved prior to use. The Dye Reagent A working solution can be stored in the dark at 4°C.

- **Standard Working Solution**
  Add 1 ml of distilled water to the Standard vial and mix thoroughly. Add 0.3 ml of this solution to 0.7 ml of distilled water to prepare a 1 ml Standard Solution with concentration 600 nmol/ml.

2. Sample

- **Cell and bacterial samples**
  Collect samples into a pre-cooled centrifuge tube and centrifuge at 10000 x g, 4 °C for 10 minutes. Discard the supernatant after centrifugation, and resuspend the pellet with 0.99 ml Assay Buffer I and 10 µl Assay Buffer II on ice. Centrifuge at 10000 x g, 4 °C for 10 minutes, discard the supernatant. Add 198 µl Assay Buffer III and 2 µl Assay Buffer II to the pellet, and sonicate at 20% power (3 seconds at 10 second intervals, repeat 30 times). Add 800 µl distilled water and aspirate to mix fully. Centrifuge at 10000 x g, 4 °C for 10 minutes, take the supernatant into a pre-cooled centrifuge tube, and keep on ice for detection.

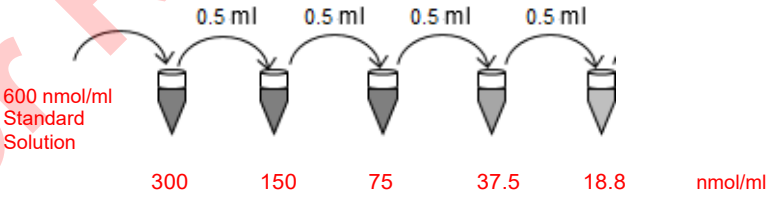
- **Tissue homogenates**
  Weigh 0.1 g tissue, add 0.99 ml of Assay Buffer I and 10 µl Assay Buffer II, and homogenize on ice by hand, using a mechanical homogenizer, or by ultrasonication. Collect into a pre-cooled centrifuge tube, and centrifuge at 10000 x g, 4 °C for 10 minutes. Discard the supernatant, and resuspend the pellet with 2 µl Assay Buffer II and 198 µl Assay Buffer III. Sonicate at 20% power (3 seconds at 10 second intervals, repeat 30 times). Add 800 µl distilled water and aspirate to mix fully. Centrifuge at 10000 x g, 4 °C for 10 minutes, take the supernatant into a pre-cooled centrifuge tube and keep on ice for detection. The protein concentration of the supernatant should be determined via the standard curve.

B. Assay Procedure

Bring all reagents to room temperature prior to use.

If the expected activity is unknown, a preliminary experiment is recommended to determine if sample dilutions or adjustments to the reaction time are required to bring the measured activity within the detection range of the kit.

1. Label 5 tubes with 300 nmol/ml, 150 nmol/ml, 75 nmol/ml, 37.5 nmol/ml and 18.8 nmol/ml. Aliquot 0.5 ml of distilled water into each tube. Add 0.5 ml of 600 nmol/ml standard solution to the 1st tube (300 nmol/ml) and mix thoroughly. Transfer 0.5 ml from the 1st tube to the 2nd tube and mix thoroughly, and so on.



2. Set the sample, standard, and blank wells on the 96 well microplate and record their positions. We recommend setting up each standard and sample in duplicate.
3. Add 10 µl of sample to the sample wells.
4. Add 90 µl of substrate to the sample wells.
5. Add 100 µl of Standard to each of the Standard wells (600 nmol/ml, 300 nmol/ml, 150 nmol/ml, 75 nmol/ml, 37.5 nmol/ml and 18.8 nmol/ml)
6. Add 100 µl of distilled water to the Blank wells
7. Add 90 µl of Dye Reagent Solution to all wells.
8. Add 10 µl of Dye Reagent Solution to all wells.
9. Tap the plate gently to mix.
10. Incubate at 37°C for 2 minutes.
11. Read and record absorbance at 450 nm.

For Reference Only

C. Calculations

One unit of SDH activity is defined as amount of enzyme required to produce 1 nmol NADH per minute.

Succinate Dehydrogenase (SDH) activity per mg of protein:

$$\mathbf{SDH\ (U/mg)} = \frac{\mathbf{C_{Standard} \times V_{Standard}}}{\mathbf{V_{Sample} \times C_{Protein} \times T}} \times \frac{\mathbf{OD_{Sample} - OD_{Blank}}}{\mathbf{OD_{Standard} - OD_{Blank}}} = \frac{\mathbf{3000}}{\mathbf{C_{Protein}}} \times \frac{\mathbf{OD_{Sample} - OD_{Blank}}}{\mathbf{OD_{Standard} - OD_{Blank}}}$$

SDH activity per g of sample:

$$\mathbf{SDH\ (U/g)} = \frac{\mathbf{C_{Standard} \times V_{Standard} \times V_{Assay}}}{\mathbf{V_{Sample} \times W}} \times \frac{\mathbf{OD_{Sample} - OD_{Blank}}}{\mathbf{OD_{Standard} - OD_{Blank}}} = \frac{\mathbf{3000}}{\mathbf{W}} \times \frac{\mathbf{OD_{Sample} - OD_{Blank}}}{\mathbf{OD_{Standard} - OD_{Blank}}}$$

SDH activity per 10^4 cells or bacteria:

$$\mathbf{SDH\ (U/10^4\ cells)} = \frac{\mathbf{C_{Standard} \times V_{Standard} \times V_{Assay}}}{\mathbf{V_{Sample} \times N}} \times \frac{\mathbf{OD_{Sample} - OD_{Blank}}}{\mathbf{OD_{Standard} - OD_{Blank}}} = \frac{\mathbf{3000}}{\mathbf{N}} \times \frac{\mathbf{OD_{Sample} - OD_{Blank}}}{\mathbf{OD_{Standard} - OD_{Blank}}}$$

Nitric Oxide concentration per ml serum or plasma:

$$\mathbf{SDH\ (U/ml)} = \frac{\mathbf{C_{Standard} \times V_{Standard}}}{\mathbf{V_{Sample}}} \times \frac{\mathbf{OD_{Sample} - OD_{Blank}}}{\mathbf{OD_{Standard} - OD_{Blank}}} = \mathbf{3000} \times \frac{\mathbf{OD_{Sample} - OD_{Blank}}}{\mathbf{OD_{Standard} - OD_{Blank}}}$$

where:

$\mathbf{C_{Protein}}$	Concentration of protein (in mg/ml)
$\mathbf{C_{Standard}}$	Concentration of highest standard (600 nmol/ml)
$\mathbf{W}$	Weight of the sample (in g)
$\mathbf{N}$	Number of cells or bacteria (× 10^4)
$\mathbf{V_{Assay}}$	Volume of Assay Buffer (1 ml)
$\mathbf{V_{Sample}}$	Volume of sample (0.01 ml)
$\mathbf{V_{Standard}}$	Volume of standard (0.1 ml)
$\boldsymbol{T}$	Reaction time (2 minutes)

For Reference Only