Succinate Dehydrogenase (SDH) Assay Kit

Catalog No.: abx298836

Size: 96 tests

Detection Range: 0.83 U/L – 65.42 U/L

Sensitivity: 0.83 U/L

Storage: Store all components at -20°C. Store the Inhibiting Reagent, Substrate A, Substrate B, and Substrate C in the dark.

Application: For detection and quantification of Succinate Dehydrogenase activity in tissue homogenates and cell lysates.

Introduction

Succinate Dehydrogenase is a key metabolic enzyme that catalyzes step 6 of the citric acid cycle, which is the principal mechanism driving respiration. Succinate Dehydrogenase catalyzes the conversion of succinate to fumarate, and is present in all eukaryotes and many bacteria. The importance of the citric acid cycle in all eukaryotic cells means that Succinate Dehydrogenase function is important in numerous research areas, ranging from human and veterinary medicine to crop science and agriculture.

Abbexa's Succinate Dehydrogenase (SDH) Assay Kit is a quick, convenient, and sensitive method for measuring and calculating Succinate Dehydrogenase activity. When the enzyme converts succinate to fumarate, the chromogenic molecule DCPIP will be oxidized. Oxidized DCPIP has an absorbance maximum at 600 nm. The intensity of the color will be proportional to the Succinate Dehydrogenase activity, which can then be calculated.

Kit components

1. 96-well microplate
2. Assay Buffer A: 2 × 50 ml
3. Assay Buffer B: 30 ml
4. Inhibiting Reagent: 2 × 0.8 ml
5. Substrate A: 2 × 1.2 ml
6. Substrate B: 2 × 1.2 ml
7. Substrate C: 1.2 ml
8. Plate sealer: 2

Materials required but not provided

1. Microplate reader (600 nm)
2. PBS (0.01 M, pH 7.4)
3. Normal saline (0.9% NaCl)
4. Double-distilled water
5. Pipette and pipette tips
6. 1.5 ml microcentrifuge tubes
7. Refrigerated centrifuge
8. Vortex mixer
9. Ice bath

Protocol

A. Preparation of samples and reagents

1. Samples

Isolate the test samples soon after collecting and analyze immediately or aliquot and store at -20°C or -80°C for long-term storage. Avoid multiple freeze-thaw cycles.

The following sample preparation methods are intended as a guide and may be adjusted as required depending on the specific samples used.

- **Tissue Homogenates:** Carefully weigh 0.1 g of tissue, and wash in ice-cold PBS (0.01 M, pH 7.4). Add the tissue into a tube containing 900 µl Assay Buffer A and 10 µl Inhibiting Reagent, kept on ice. Mix fully, then homogenize manually, using a mechanical homogenizer at 4°C. Centrifuge at 600 × g for 5 minutes at 4°C. Take the supernatant into a new tube, and then centrifuge at 15,000 × g for 10 minutes at 4°C.

  Resuspend the precipitate (which contains extracted mitochondria) with 200 µl Assay Buffer B and 2 µl Inhibiting Reagent, kept on ice. Mix fully, then homogenize by ultrasonication for 5 minutes at 4°C. Centrifuge at 15,000 × g for 10 minutes at 4°C. Carefully take the supernatant for analysis.

- **Cell Lysates:** Harvest 2×10^6 cells, and wash in ice-cold PBS (0.01 M, pH 7.4). Add the cells into a tube containing 400 µl Assay Buffer A and 4 µl Inhibiting Reagent, kept on ice. Mix fully, then homogenize by ultrasonication at 4°C. Centrifuge at 600 × g for 5 minutes at 4°C. Take the supernatant into a new tube, and then centrifuge at 15,000 × g for 10 minutes at 4°C.

  Resuspend the precipitate (which contains extracted mitochondria) with 200 µl Assay Buffer B and 2 µl Inhibiting Reagent, kept on ice. Mix fully, then homogenize by ultrasonication for 5 minutes at 4°C. Centrifuge at 15,000 × g for 10 minutes at 4°C. Carefully take the supernatant for analysis.

Note: To calculate Succinate Dehydrogenase activity in tissue homogenates and cell lysates using the formula in section **C. Calculation of Results**, the total protein concentration of the supernatant must be determined separately.

It is recommended to carry out a preliminary experiment to determine the optimal dilution factor of samples before carrying out the formal experiment. Dilute samples into different concentrations with Assay Buffer B, then carry out the assay procedure. The recommended dilution factor for different samples is as follows (for reference only):

Sample Type	Dilution Factor
10% Rat liver tissue homogenate	1
10% Rat heart tissue homogenate	1
10% Rat kidney tissue homogenate	1
10% Mouse liver tissue homogenate	1
10% Mouse heart tissue homogenate	1
10% Mouse kidney tissue homogenate	1

Note:

- Total protein concentration of the prepared homogenate should be tested on the same day as sample preparation.
- Keep the indicated reagents in the dark whenever possible during sample preparation.
- To prevent sample degradation due to delays during preparation, it is recommended to test no more than 8 samples simultaneously.
- Fresh samples or recently obtained samples are recommended to prevent degradation and denaturalization that may lead to erroneous results.
- Lysis buffers may interfere with the kit. It is therefore recommended to use mechanical lysis methods for cell lysates and tissue homogenates.

2. Reagents

- **Working Substrate Solution:** For each well tested, prepare 190 µl of Working Substrate Solution by mixing 140 µl of Assay Buffer A, 20 µl of Substrate A, 20 µl of Substrate B, and 10 µl of Substrate C.

  The Working Substrate Solution should be prepared just before use, and should not be stored.

Note:

- Allow all reagents to equilibrate to room temperature before use.

B. Assay Procedure

1. Mark positions on the 96-well microplate for each sample, and blank. *It is strongly recommended to prepare all the wells in duplicate.*
2. Add 20 µl of Assay Buffer B to the blank wells.
3. Add 20 µl of sample to the corresponding sample wells.
4. Add 190 µl of Working Substrate Solution to all wells.
5. Tap or shake the plate gently for 5 seconds to mix the well contents.
6. Measure the OD of each well with a microplate reader at 600 nm. Record these values as A1.
7. Leave the plate to stand at room temperature for 3 minutes.
8. Measure the OD of each well with a microplate reader at 600 nm. Record these values as A2.

C. Calculation of Results

For each sample and blank, calculate the mean absorbance at A1 and A2, then find the mean change in absorbance for that sample or control by calculating $\overline{A_1} - \overline{A_2}$.

The activity of Succinate Dehydrogenase in each sample well can be derived with the following formulae:

1. Tissue Homogenate and Cell Lysate samples:

One unit of Succinate Dehydrogenase activity is defined as the amount required for 1 g of tissue or cell total protein to produce 1 µmol of product at room temperature.

$$\text{Succinate Dehydrogenase (U/g protein)} = F \times 1000 \times \frac{(OD_{Sample} - OD_{Blank}) \times V_{Total}}{21.8 \times t \times V_{Sample} \times C_{Protein}}$$

where:

OD_{Sample}	Change in OD value of sample (A1 – A2)
OD_{Blank}	Change in OD value of blank (A1 – A2)
V_{Total}	Volume of the reaction system (0.21 ml)
V_{Sample}	Volume of the sample added (0.02 ml)
$C_{Protein}$	Total concentration of protein in the sample (g protein/L)
t	Reaction time (3 minutes)
F	Dilution factor of the sample

Technical Support

For troubleshooting and technical assistance, please contact us at support@abbexa.com.