Signosis, Inc.
Innovative Plate Assay Solutions

Succinate Dehydrogenase Assay Kit

Catalog # EA-7022 **(For Research Use Only)**

Introduction

The Succinate Dehydrogenase (SDH) Assay Kit utilizes DCPIP, a blue-colored redox dye, to measure SDH activity in samples. When SDH converts succinate to fumarate, it generates $FADH_2$, which transfers electrons to an electron acceptor. In this assay, the electrons are transferred to DCPIP, which becomes reduced and changes color. This color change can be measured with a spectrophotometer at an absorbance of 600 nm.

Materials Required but Not Provided

- PBS
- 96-well clear microplate for absorbance reading or 96-well black microplate with clear bottom for fluorescence reading
- Microplate reader capable of measuring absorbance at 600 nm

Materials Provided

- 10mM FAD (-80ºC)
- Succinate Substrate (-20ºC)
- Transport Reagent (-20ºC)
- 20mM DCPIP (-20ºC)

****Spin down small tubes before starting experiment. ****

Plasma Sample Preparation

1. Centrifuge citrated or EDTA-collected blood at 4°C (1,000 x *g* for 10 minutes) to separate plasma from erythrocytes. Alternatively, blood collected without anticoagulant can be centrifuged to collect serum.
2. Transfer the plasma layer to a new tube without disturbing the buffy layer.
3. The plasma may be assayed directly or stored away at -80°C.

Cell Sample Preparation

1. Wash the cells once with PBS before lysing the cells.
2. For a 96-well culture plate, add 40 µL of lysis buffer to each well and incubate at room temperature for 10 minutes.
3. Pipette the lysis buffer up and down to detach the cells and transfer the cell lysates into a new tube.
4. If necessary, homogenize the cell lysates with a sonicator.
5. The cell lysates may be assayed directly or stored at -80°C.
6. Use PBS to dilute the cell sample to the appropriate concentration, if necessary.

Tissue Sample Preparation

1. Weigh tissue sample and add 1 mL of lysis buffer per 100mg of tissue.
2. Homogenize the tissue samples with a tissue grinder.
3. If necessary, further homogenize the tissue samples with a sonicator.
4. Centrifuge the sample at 10,000 RPM for 5 minutes to pellet the tissue debris.
5. Collect the supernatant and measure the protein concentration of the supernatant. The tissue sample can be assayed directly or stored at -80°C.
6. Use PBS to dilute the tissue sample to the appropriate concentration, if necessary.

SDH Activity Measurement

1. Reaction mix preparation: calculate the amount of each reagent needed to make the reaction mix according to the table below.

Component	Reaction Mix (per well/sample)
10mM FAD	0.05 µL
Succinate Substrate	0.05 µL
Transport Reagent	0.5 µL
20mM DCPIP	0.5 µL
PBS	48.9 µL
Total	50 µL

2. Add 50 µL of reaction mix to each well of the plate.
3. Add 50 µL of sample to each well with reaction mix and mix thoroughly.
4. Measure the absorbance of the plate at 600 nm. Obtain kinetic data for SDH activity by making multiple readings of the plate. Start at one-minute intervals and increase the interval time as the signal begins to stabilize.