Product Information

Succinate Dehydrogenase Activity Colorimetric Assay Kit

I. Kit Contents:

Components	K2210-100 100 assays	Cap Color	Part Number
SDH Assay Buffer	25 ml	WM	K2210-C-1
SDH Substrate Mix (Lyophilized)	1 vial	Blue	K2210-C-2
SDH Probe	0.2 ml	Red	K2210-C-3
DCIP Standard (2 mM)	0.4 ml	Yellow	K2210-C-4
SDH Positive Control (Lyophilized)	1 vial	Orange	K2210-C-5

II. Introduction:

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

The Succinate Dehydrogenase Activity Colorimetric Assay Kit provides a sensitive, fast and simple way for detection of SDH activity in various samples based on colorimetric method. In the assay, SDH converts succinate to fumarate and transfers the electron to an artificial electron acceptor (SDH Probe), which transforms the color from blue to a colorless product (depending upon the sample enzymatic activity). The assay is high-throughput adaptable. The kit can detect less than 0.1mU SDH activity in a variety of samples.

III. Application:

Measurement of Succinate Dehydrogenase Activity in various tissues/cells.

Analysis of citric acid cycle.

VI. Sample Type:

Animal tissues: heart, liver, muscle, etc.

Purified mitochondria.

Cell culture: Adherent or suspension cells.

V. User Supplied Reagents and Equipment:

96-well plate with flat clear bottom.

Multi-well spectrophotometer (ELISA reader).

VI. Reagent Preparation and Storage Conditions:

SDH Assay Buffer: Warm to room temperature before use. Store at either 4℃ or -20℃.

SDH Substrate Mix: Reconstitute with 220 µl dH_2O. Pipette up and down to dissolve completely. Aliquot and store at -20℃. Avoid repeated freeze/thaw. Keep on ice while in use.

SDH Probe and DCIP Standard: Warm to room temperature before use. Store at -20℃.

SDH Positive Control: Reconstitute with 100 µl SDH Assay Buffer. Aliquot and store at -80℃. Avoid repeated freeze/thaw. Keep on ice while in use.

VII. Reagent Preparation and Storage Conditions:

Acetate Enzyme Mix: Reconstitute with 220 µl Acetate Assay Buffer. Pipette up and down to dissolve completely. Aliquot and store at -20℃. Avoid repeated freeze thaw. Keep on ice while in use. Stable for 2 months.

ATP: Reconstitute with 220 µl dH_2O. Pipette up and down to dissolve completely. Aliquot and store at -20℃. Keep on ice while in use. Stable for 2 months.

Acetate Substrate Mix: Dissolve with 220 µl Acetate Assay Buffer. Pipette up and down to dissolve completely. Stable for 2 months at -20℃.

Probe: Reconstitute with 220 µl dH_2O. Pipette up and down to dissolve completely. Keep on ice while in use. Stable for 2 months at -20℃.

Acetate Standard: Reconstitute with 100 µl dH_2O to generate 100 mM (100 nmol/µl) Acetate Standard solution. Keep on ice while in use. Store at -20℃. Use within two months.

VIII. Assay Protocol:

1. Sample Preparation: Rapidly homogenize tissue (10 mg) or cells (1 x 10^6) with 100 µl ice cold SDH Assay Buffer, and keep on ice for 10 min. Centrifuge at 10,000 x g for 5 min. and transfer the supernatant to a fresh tube. Add 5 - 50 µl sample per well & adjust the volume to 50 µl with SDH Assay Buffer. To check SDH activity in mitochondria, isolate the mitochondria from fresh tissue or cells using Mitochondria Isolation Kit for Tissue and Cultured Cells. Add 5 - 50 µl isolated mitochondria per well, adjust the volume to 50 µl/well with SDH Assay Buffer. For the SDH positive control, take 10 - 20 µl of SDH Positive Control into desired well(s) and adjust the final volume to 50 µl with SDH Assay Buffer.

Note:

For unknown samples, we suggest doing a pilot experiment & testing several doses to ensure the readings are within the Standard Curve range.

2. Standard Curve Preparation: Add 0, 4, 8, 12, 16 & 20 µl of the 2 mM DCIP Standard into a series of wells in 96-well plate to generate 0, 8, 16, 24, 32, and 40 nmol/well of DCIP Standard. Adjust the volume to 100 µl/well with SDH Assay Buffer.

3. Reaction Mix: Mix enough reagents for the number of assays (samples and Positive Control) to be performed. For each well, prepare 50 µl Reaction Mix containing:

SDH Assay Buffer	46 µl
SDH Substrate Mix	2 µl
SDH Probe	2 µl

Add 50 µl of the Reaction Mix to each well containing the samples and positive control, mix well.

4. Measurement: Measure the absorbance immediately at 600 nm in kinetic mode for 10 - 30 min. at 25℃.

Note: Incubation time depends on the succinate dehydrogenase activity in samples. We recommend measuring the OD in kinetic mode, and choosing two time points (T1 & T2) in the linear range to calculate the succinate dehydrogenase activity of the samples. The DCIP Standard Curve can be read in Endpoint mode (i.e., at the end of the incubation time).

5. Calculation: Subtract 0 Standard reading from all readings. Plot the DCIP Standard Curve. Calculate the succinate dehydrogenase activity of the test sample: ΔOD = A1 – A2. Apply the ΔOD to the DCIP Standard Curve to get B nmol of DCIP reduced during the reaction time (ΔT = T2 - T1).

Sample Succinate Dehydrogenase Activity = B/(ΔT X V) x Dilution Factor = nmol/min/µl = mU/µl = U/ml

Where: B = amount of reduced DCIP from Standard Curve (nmol)

ΔT = reaction time (min.)

V = sample volume added into the reaction well (µl)

D = Dilution Factor

Unit Definition: One unit of SDH is the amount of enzyme that generates 1.0 µmol of DCIP per min. at pH 7.2 at 25℃.

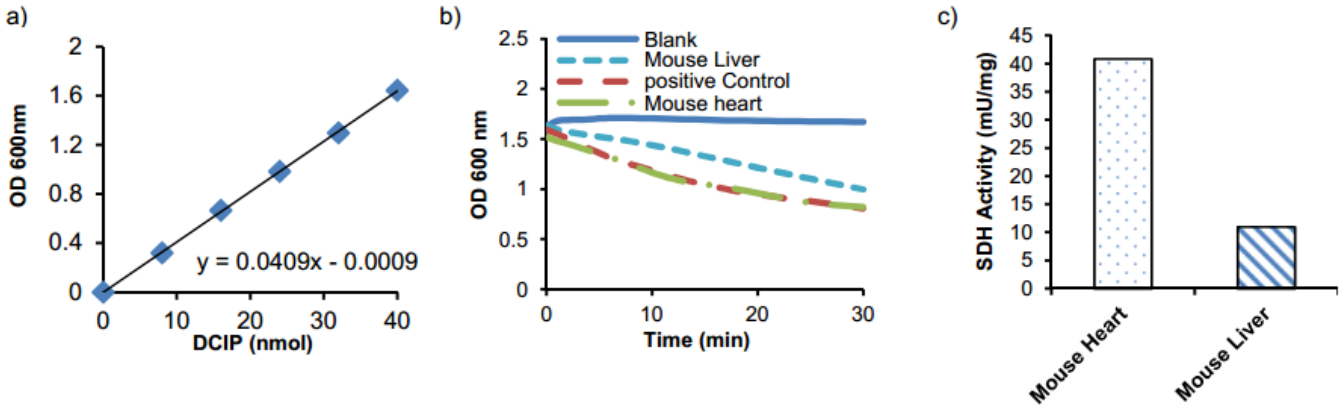

Figure: (a) DCIP Standard Curve (b) & (c) Measurement of Succinate Dehydrogenase Activity in Positive Control (22 µg) & mitochondria isolated from mouse heart (24 µg) & liver (70 µl).

Frequently Asked Questions

1. Can this kit measure SDHA activity without the other components of Complex II?

We have not distinguished SDHA activity from the activity of the SDH complex while testing this kit. It measures the conversion of succinate to fumarate by SDH.

2. Can frozen samples be used with this assay?

Fresh samples are always preferred over frozen samples. However, frozen samples can also be used, provided, they were frozen right after isolation, were not freeze thawed multiple time (for which we recommend aliquoting the samples before freezing) and have been frozen for relatively short periods.

3. Is it possible to use a different wavelength than recommended for the final analysis?

It is always recommended to use the exact recommended wavelength for the most efficient results. However, most plate readers have flexibility in their band width of detection in increments of +/- 10 nm. Depending on this flexibility range, you can deviate from the recommended wavelengths within limits.

4. What is the exact volume of sample required for this assay?

There is no specific volume we can recommend for the amount any sample to be used since it is completely sample concentration and quality based. It is recommended to do a pilot expt with multiple sample volumes to determine the optimal volume which gives a reading within the linear range of the standard curve. Please refer to the citations for this product to see what other clients have used with similar sample types.

For research use only! Not to be used in humans.

Our promise

If the product does not perform as described on this datasheet, we will offer a refund or replacement. For more details, please visit http://www.apexbt.com/ or contact our technical team.

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