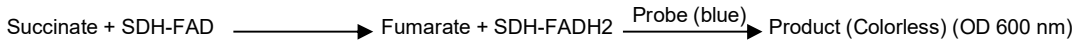
Succinate Dehydrogenase Activity Colorimetric Assay Kit (#BN00884)

(Catalog #BN00884; 100 assays; Store at -20°C)

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. Assay Genie's Succinate Dehydrogenase Activity Assay kit is rapid, simple and high-throughput adaptable. In this assay, Succinate dehydrogenase converts succinate to fumarate, and transfers the electron to an artificial electron acceptor (Probe), which changes the color from blue to a colorless product (depending upon the sample enzymatic activity). This assay kit can detect less than 0.1mU Succinate Dehydrogenase Activity in a variety of samples.



II. Application:

- Measurement of Succinate Dehydrogenase Activity in various tissues/cells.
- Analysis of citric acid cycle

III. Sample Type:

- Animal tissues: heart, liver, muscle, etc.
- Purified mitochondria
- Cell culture: Adherent or suspension cells

IV. Kit Contents:

Components	BN00884	Cap Code	Part Number
SDH Assay Buffer	25 ml	WM	BN00884-1
SDH Substrate Mix (Lyophilized)	1 vial	Blue	BN00884-2
SDH Probe	0.2 ml	Red	BN00884-3
DCIP Standard (2 mM)	0.4 ml	Yellow	BN00884-4
SDH Positive Control (Lyophilized)	1 vial	Orange	BN00884-5

V. User Supplied Reagents and Equipment:

- 96-well plate with flat clear bottom
- Multi-well spectrophotometer (ELISA reader)

VI. Storage and Handling:

Store kit at -20°C, protected from light. Warm Assay Buffer to room temperature before use. Briefly centrifuge small vials prior to opening. Read the entire protocol before performing the assay.

VII. Reagent Preparation and Storage Conditions:

- **SDH Assay Buffer:** Warm to room temperature before use. Store at either 4ºC or -20ºC.
- **SDH Substrate Mix**: Reconstitute with 220 µl dH_2O. Pipette up and down to dissolve completely. Aliquot and store at -20°C. Avoid repeated freeze/thaw. Keep on ice while in use.
- **SDH Probe and DCIP Standard:** Warm to room temperature before use. Store at -20ºC.
- **SDH Positive Control**: Reconstitute with 100 µl SDH Assay Buffer. Aliquot and store at -80°C. Avoid repeated freeze/thaw. Keep on ice while in use.

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 Assay Genie's 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:

	Reaction Mix
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°C.

Note: Incubation time depends on the succinate dehydrogenase activity in samples. We recommend measuring the OD in kinetic mode, and choosing two time points (T_1 & T_2) 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: $\Delta OD = A_1 - A_2$. Apply the ΔOD to the DCIP Standard Curve to get B nmol of DCIP reduced during the reaction time ($\Delta T = T_2 - T_1$).

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)
Δ**T** = 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°C.

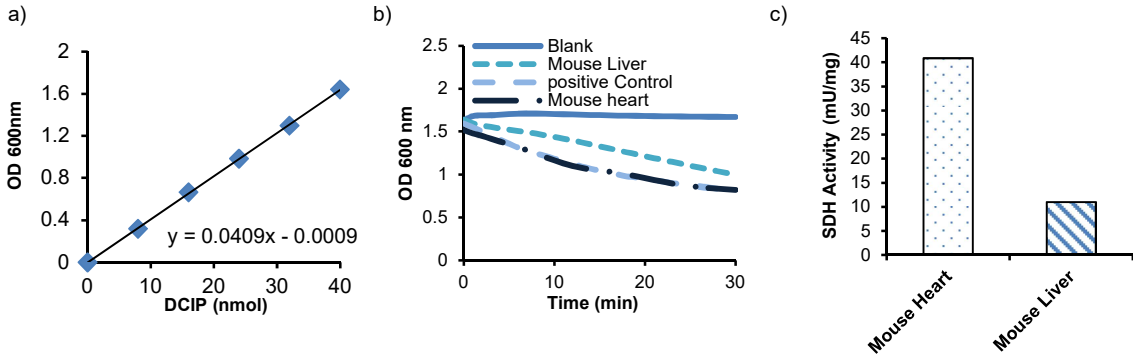


Figure: (a) DCIP Standard Curve (b) & (c) Measurement of Succinate Dehydrogenase Activity in Positive Control (22 µg) & mitochondria isolated from mouse heart (24 µg) & liver (70 µl).

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