Succinyl-CoA Synthetase Activity Assay Kit (Colorimetric)

Catalog Number KA4557

100 assays

Version: 02

Intended for research use only

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Introduction

Background

Succinyl-CoA Synthetase (SCS, also called Succinyl-CoA ligase, Succinate Thiokinase) (EC 6.2.1.5) is a critical enzyme in the citric acid cycle and an important metabolic intermediate for porphyrin, heme and ketone body biosynthesis. It is located in the mitochondrial matrix and is a heterodimer composed of one α and one β subunit. In humans, Succinyl-CoA Synthetase deficiency causes the build-up of lactic acid leading to lactic acidosis, which can be fatal in infants. Measurement and analysis of SCS activity is useful for both mechanistic studies as well as for diagnostic purposes. In BioVision's Succinyl-CoA Synthetase Activity Assay, SCS converts succinate into succinyl-CoA in the presence of ATP and CoA. Succinyl-CoA reacts with the Developer to form a colored product with strong absorbance at 450 nm. This assay kit is simple, sensitive, and high-throughput adaptable. It can detect less than 0.1 mU of Succinyl-CoA Synthetase activity in a variety of samples.

✓ Application

- Measurement of Succinyl-CoA Synthetase activity in various tissues/cells
- Analysis of cell signaling pathway

✓ Sample Type

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

General Information

Materials Supplied

List of component

Component	Amount
SCS Assay Buffer	25 mL
SCS Substrate Mix (Lyophilized)	1 vial
SCS Enzyme Mix (Lyophilized)	1 vial
SCS Developer (Lyophilized)	1 vial
NADH Standard (Lyophilized)	1 vial
SCS Positive Control (Lyophilized)	1 vial

Storage Instruction

Store the kit at -20°C, protected from light.

Materials Required but Not Supplied

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

Precaution

For Research Use Only. Not to be used on humans.

Assay Protocol

Reagent Preparation

Briefly centrifuge small vials prior to opening. Read the entire protocol before performing the assay.

- ✓ SCS Assay Buffer: Warm to room temperature before use. Store at either 4ºC or -20ºC.
- ✓ SCS Substrate Mix and SCS Developer: Reconstitute with 220 µL dH_2O. Pipette up and down to dissolve completely. Store at -20°C.Keep on ice while in use. Use within two months.
- ✓ SCS Enzyme Mix: Reconstitute with 220 µL SCS Assay Buffer. Gently pipette up and down to dissolve completely. Store at -20°C. Use within two months.
- ✓ NADH Standard: Reconstitute with 400 µL dH_2O to generate 1.25 mM NADH Standard solution. Aliquot and store at -20°C. Keep on ice while in use. Use within two months.
- ✓ SCS Positive Control: Reconstitute with 100 µL SCS Assay Buffer and mix thoroughly. Aliquot and store at -70°C. Keep on ice while in use. Use within two months.

Sample Preparation

Rapidly homogenize tissue (10 mg) or cells (1 x 10^6) with 100 µL ice cold SCS 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 SCS Assay Buffer. To check SCS activity in mitochondria, isolate the mitochondria from fresh tissue or cells using Mitochondria Isolation Kit for Tissue and Cultured Cells. Add 5-50 µL of isolated mitochondria per well and adjust the volume to 50 µL with SCS Assay Buffer. For the SCS Positive Control, add 1-10 µL of SCS Positive Control into desired well(s) and adjust the volume to 50 µL with SCS Assay Buffer.

Note:

1. *For unknown samples, we suggest doing a pilot experiment & testing several doses to ensure the readings are within the Standard Curve range.*
2. *For samples exhibiting background, prepare parallel sample well(s) as background control.*

Assay Procedure

- ✓ NADH Standard Curve

  Add 0, 2, 4, 6, 8 and 10 µL of 1.25 mM NADH Standard into a series of wells in 96 well plate to generate 0, 2.5, 5.0, 7.5, 10 and 12.5 nmol/well of NADH Standard. Adjust the volume to 50 µL per well with SCS Assay Buffer.

✓ Reaction Mix

Mix enough reagents for the number of assays to be performed. For each well, prepare 50 µL Reaction Mix containing:

	Reaction Mix	*Background Control Mix
SCS Assay Buffer	44 µL	46 µL
SCS Substrate Mix	2 µL	-
SCS Enzyme Mix	2 µL	2 µL
SCS Developer	2 µL	2 µL

Mix and add 50 µL of the Reaction Mix to each well containing the Standard, Positive Control, and test samples.

* For samples, which require correction due to significant background, add 50 µL of Background Control Mix to sample background control well(s) and mix well.

✓ Measurement

Measure the absorbance (OD 450 nm) in kinetic mode for 10-30 min at 25°C.

Note: Incubation time depends on the Succinyl-CoA Synthetase activity in samples. We recommend measuring the OD in a kinetic mode, and choosing two time points (T_1 & T_2) in the linear range to calculate the Succinyl-CoA Synthetase activity. The NADH Standard Curve can be read in the endpoint mode (i.e. at the end of the incubation time).

Data Analysis

Calculation of Results

Subtract 0 Standard reading from all readings. Plot the NADH Standard Curve. If sample background control reading is significant, subtract the background control reading instead, from its paired sample reading. Calculate the Succinyl-CoA Synthetase activity of the test sample: $\Delta OD = A_2 - A_1$. Apply the ΔOD to the NADH Standard Curve to get B nmol of NADH generated during the reaction time ($\Delta T = T_2 - T_1$).

Sample Succinyl-CoA Synthetase Activity = B/(ΔT X V) x Dilution Factor = nmol/min/µL = mU/µL = U/mL

Where: B = NADH amount from Standard Curve (nmol)

ΔT = reaction time (min)

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

D = dilution factor

Succinyl-CoA Synthetase activity can also be expressed as mU/mg of protein.

✓ Unit Definition

One unit of Succinyl-CoA Synthetase is the amount of enzyme that generates 1.0 µmol of NADH per min at pH 7.4 at 25°C.

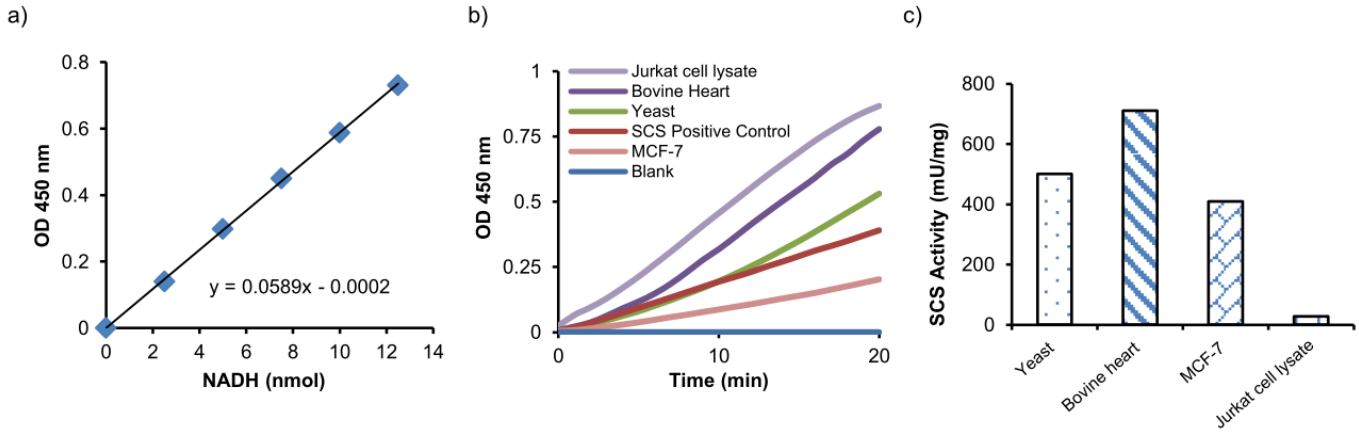

Figure: (a) NADH Standard Curve; (b) Succinyl-CoA Synthetase activity in mitochondria prepared from bovine heart, yeast (P. pastoris) and MCF-7 cells and in Jurkat cell lysate; (c) Succinyl-CoA Synthetase specific activity in mitochondria prepared from yeast (P. pastoris, 1.14 µg), bovine heart (1.1 µg) and MCF-7cells (0.5 µg), and in Jurkat cell lysate (25 µg). Assays were performed following the kit protocol.