CREATIVE BIOMART®
Assay Kit

Succinyl-CoA Synthetase Activity Colorimetric Assay Kit

Product Information

Cat

Kit-0777

Common Name

SCS

Cat.No.

Kit-0777

Product Overview

Succinyl-CoA Synthetase Activity Assay Kit (Colorimetric) is used to measure Succinyl-CoA Synthetase activity.

Description

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 a 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 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.

Applications

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

Usage

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Email:info@creative-biomart.org
Fax:1-631-938-8127
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For research use only (RUO)

Storage

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

Kit Components

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

Materials Required but Not Supplied

96-well clear plate with flat bottom
Multi-well spectrophotometer

Target Species

Mammals

Detection method Colorimetric

Compatible Sample Types

Animal tissues: heart, liver, muscle, etc.Cell culture: adherent or suspension cellsPurified mitochondria

Preparation

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 4°C or -20°C.
SCS Substrate Mix and SCS Developer: Reconstitute with 220 μL dH2O. 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.

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NADH Standard: Reconstitute with 400 μL dH2O 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 linear range.
2. For samples exhibiting background, prepare parallel sample well(s) as background control.

Assay Protocol

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

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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 25oC.
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 (T1 & T2) 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).

Analysis

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: ΔOD = A2 – A1. Apply the ΔOD to the NADH
Standard Curve to get B nmol of NADH generated during the reaction time (ΔT = T2- T1).
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 7.0 at 25°C.

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