Succinyl-CoA Synthetase Activity Colorimetric Assay Kit

Cat. No. Kit-0777 **Lot. No.** (See product label)

SPECIFICATION

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 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 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	For research use only (RUO)
Storage	Store the kit at -20°C, protected from light.
Kit Components	SCS Assay Buffer 25 mL

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	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 4ºC or -20ºC. 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. 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 µ SCS Assay Buffer and mix thoroughly.

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	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 µ 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 µ 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 µ 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 µ 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 µ Reaction Mix containing: Reaction Mix *Background Control Mix SCS Assay Buffer 44 µ 46 µ SCS Substrate Mix 2 µ - SCS Enzyme Mix 2 µ 2 µ

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	SCS Developer 2 µ 2 µ Mix and add 50 µ 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 µ 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 (µ) 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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