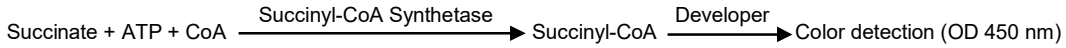
Succinyl-CoA Synthetase Activity Colorimetric Assay Kit (BN00824)

(Catalog # BN00824; 100 assays; Store at -20°C)

I. Introduction:

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 Assay Genie'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.



II. Application:

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

III. Sample Type:

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

IV. Kit Contents:

Components	BN00824	Cap Code	Part Number
SCS Assay Buffer	25 ml	WM	BN00824-1
SCS Substrate Mix (Lyophilized)	1 vial	Blue	BN00824-2
SCS Enzyme Mix (Lyophilized)	1 vial	Green	BN00824-3
SCS Developer (Lyophilized)	1 vial	Red	BN00824-4
NADH Standard (Lyophilized)	1 vial	Yellow	BN00824-5
SCS Positive Control (Lyophilized)	1 vial	Orange	BN00824-6

V. User Supplied Reagents and Equipment:

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

VI. Storage Conditions and Reagent Preparation:

Store kit at –20°C, protected from light. 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.

VII. Succinyl-CoA Synthetase Activity Assay Protocol:

1. **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 Assay Genie's 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:

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

   b. For samples exhibiting background, prepare parallel sample well(s) as background control.

2. **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.

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

4. **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 kinetic mode, and choosing two time points (T_1 & T_2) in the linear portion of the time course to calculate the Succinyl-CoA Synthetase activity. The NADH 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 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 samples: $\Delta OD = A_2 - A_1$. 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).
Δ**T** = 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 will generate 1.0 µmol of NADH per min. at pH 7.4 at 25°C.

a) b) c)

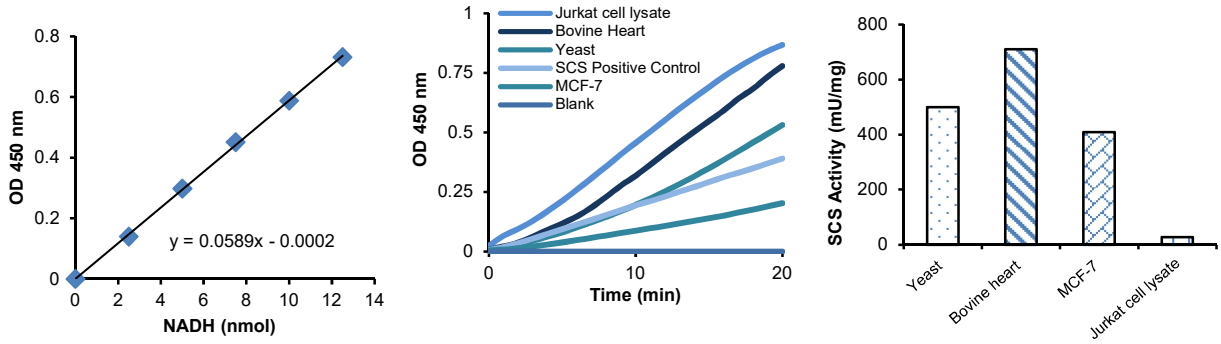


Figure: (a) NADH Standard Curve; (b) Succinyl-CoA Synthetase activity in mitochondria prepared from bovine heart, yeast (*P. pastoris)* and MCF-7 cells and in Jurkat cell lysate; (c) Succinyl-CoA Synthetase specific activity in mitochondria prepared from yeast (*P. pastoris,* 1.14 µg), bovine heart (1.1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

FOR RESEARCH USE ONLY! Not to be used on humans.