Succinyl-CoA Synthetase (SCS) Assay Kit

Catalog No.: abx294437

Size: 96 tests

Detection Range: 0.09 U/L – 20.72 U/L

Sensitivity: 0.09 U/L

Storage: Store all components at -20°C in the dark.

Application: For detection and quantification of Succinyl-CoA Synthetase activity in tissue homogenates and cell samples.

Introduction

Succinyl-CoA Synthetase catalyzes the reversible reaction of succinyl-CoA to succinic acid. It is a key enzyme in the citric acid cycle.

Abbexa's Succinyl-CoA Synthetase Assay Kit is a quick, convenient, and sensitive method for measuring and calculating Succinyl-CoA Synthetase activity. Succinyl-CoA Synthetase converts succinyl-CoA to succinic acid. This reaction produces chromogenic substances which have an absorbance maximum of 450 nm. The intensity of the color is proportional to the Succinyl-CoA Synthetase activity, which can then be calculated.

Kit components

1. 96-well microplate
2. Buffer Solution: 24 ml
3. Enzyme Solution: 1 ml
4. Accelerating Solution: 1 ml
5. Substrate Solution: 1.6 ml
6. Chromogenic Reagent: 2 × 1.5 ml
7. Co-factor Solution: 5 ml
8. Standard: 2 vials
9. Plate sealer: 2

Materials required but not provided

1. Microplate reader (450 nm)
2. Double-distilled water
3. PBS (0.01 M, pH 7.4)
4. Pipette and pipette tips
5. 1.5 ml microcentrifuge tubes
6. Centrifuge
7. Vortex mixer
8. Sonicator
9. Mechanical homogenizer

Protocol

A. Preparation of samples and reagents

1. Samples

Isolate the test samples soon after collecting and analyze immediately or aliquot and store at -20°C or -80°C for long-term storage. Avoid multiple freeze-thaw cycles.

The following sample preparation methods are intended as a guide and may be adjusted as required depending on the specific samples used.

- **Cell Samples:** Collect at least 1 × 10^6 cells and wash with cold PBS (0.01 M, pH 7.4). Per 1 × 10^6 cells, add 200 µl of PBS (0.01 M, pH 7.4) and homogenize using a sonicator. Centrifuge at 10,000 × g for 10 minutes at 4°C. Collect the supernatant and keep on ice for detection.
- **Tissue Homogenates:** Carefully weigh at least 20 mg of tissue and wash with cold PBS (0.01 M, pH 7.4). Per 20 mg of tissue, add 180 µl of PBS (0.01 M, pH 7.4) and homogenize using a mechanical homogenizer at 4°C. Centrifuge at 10,000 × g for 10 minutes at 4°C. Collect the supernatant and keep on ice for detection.

  Note: To calculate Succinyl-CoA Synthetase activity in tissue homogenates and cell samples using the formula in section **C. Calculation of Results**, the total protein concentration of the supernatant must be determined separately **(abx097193)**.

It is recommended to carry out a preliminary experiment to determine the optimal dilution factor of samples before carrying out the formal experiment. Dilute samples into different concentrations with PBS (0.01 M, pH 7.4), then carry out the assay procedure. The recommended dilution factor for different samples is as follows (for reference only):

Sample Type	Dilution Factor
10% Rat heart tissue homogenate	1
10% Rat liver tissue homogenate	1
10% Rat kidney tissue homogenate	1
10% Mouse brain tissue homogenate	1
1 × 10^6 4T1 cells	1
1 × 10^6 Molt-4 cells	1
1 × 10^6 Jurkat cells	1

Note:

- Keep the sample on ice while in use. Samples should be tested within 4 hours of preparation.
- Fresh samples or recently obtained samples are recommended to prevent degradation and denaturalization that may lead to erroneous results.

- Lysis buffers may interfere with the kit. It is therefore recommended to use mechanical lysis methods for cell lysates and tissue homogenates.

2. Reagents

- **Reaction Working Solution:** Prepare enough Reaction Working Solution for amount of test wells used. Prepare the Reaction Working Solution by mixing Accelerating Solution, Enzyme Solution, Substrate Solution, Co-factor Solution, and Buffer Solution in a ratio of 1 : 1 : 2 : 7 : 26 volume to volume.

  For example, add 5 µl of Accelerating Solution, 5 µl of Enzyme Solution, 10 µl of Substrate Solution, and 35 µl of Co-factor Solution to 130 µl of Buffer Solution and mix thoroughly to prepare 185 µl of Reaction Working Solution. Keep on ice and avoid exposure to light. Use within 24 hours.

- **Standard Solution (1 mmol/L):** Dissolve one vial of Standard with 1 ml of double-distilled water and mix thoroughly. Keep on ice and avoid exposure to light. Use within 24 hours.

- **Standards:** Label 7 tubes with 0.5 mmol/L, 0.4 mmol/L, 0.35 mmol/L, 0.3 mmol/L, 0.25 mmol/L, 0.2 mmol/L, and 0.1 mmol/L. Add 100 µl, 80 µl, 70 µl, 60 µl, 50 µl, 40 µl, and 20 µl of Standard Solution (1 mmol/L) to the 0.5 mmol/L, 0.4 mmol/L, 0.35 mmol/L, 0.3 mmol/L, 0.25 mmol/L, 0.2 mmol/L, and 0.1 mmol/L tubes respectively, followed by 100 µl, 120 µl, 130 µl, 140 µl, 150 µl, 160 µl, and 180 µl of double-distilled water, to prepare Standard Dilutions with concentrations 0.5 mmol/L, 0.4 mmol/L, 0.35 mmol/L, 0.3 mmol/L, 0.25 mmol/L, 0.2 mmol/L, and 0.1 mmol/L. Pipette gently up and down or swirl with a pipette tip to mix thoroughly. These volumes are summarized in the following table:

Standard Dilution (mmol/L)	**0.5**	**0.4**	**0.35**	**0.3**	**0.25**	**0.2**	**0.1**
1 mmol/L Standard (µl)	100	80	70	60	50	40	20
Double-distilled water (µl)	100	120	130	140	150	160	180

For the blank, or 0 mmol/L standard, use pure double-distilled water. The volume of each standard will be 200 µl.

Note:

- Allow all reagents to equilibrate to room temperature before use.

B. Assay Procedure

1. Assign and record the positions for each standard and sample well.
2. Add 20 µl of prepared standard dilutions to the corresponding standard wells.
3. Add 20 µl of sample to the sample wells.
4. Add 185 µl of Buffer Solution to the standard wells.

5. Add 185 µl of Reaction Working Solution to the sample wells.
6. Add 20 µl of Chromogenic Reagent to each well.
7. Mix fully by tapping the plate gently, then measure the OD values of each well at 450 nm. Record these as A_1 (For example, $A_{1\ Sample}$ and $A_{1\ Standard}$).
8. Incubate at room temperature for 20 minutes.
9. Measure the OD values of each well at 450 nm. Record these as A_2 (For example, $A_{2\ Sample}$ and $A_{2\ Standard}$).

C. Calculation of Results

Plot the standard curve, using the averages of the duplicate OD values for the standard dilutions (adjusted for the blank) on the y-axis, and their respective concentrations on the x-axis. The curve should form a straight line, described by the formula $y = ax + b$. Based on this curve, the activity of Succinyl-CoA Synthetase in each sample well can be derived with the following formula:

1. Tissue Homogenates and Cell Samples:

One unit of Succinyl-CoA Synthetase activity is defined as the amount required for 1 g of sample protein to produce 1 µmol of product per minute at 25°C.

$$\text{Succinyl-CoA Synthetase activity (U/g protein)} = \mathbf{F} \times \mathbf{1000} \times \frac{(\mathbf{A_{2\ Sample}} - \mathbf{A_{1\ Sample}} - \mathbf{b})}{\mathbf{a} \times \mathbf{t} \times \mathbf{C_{Protein}}}$$

where:

$A_{2\ Sample}$	OD value of sample after reaction
$A_{1\ Sample}$	OD value of sample before reaction
$C_{Protein}$	Concentration of protein in sample (mg/ml)
a	Gradient of the standard curve ($y = \boldsymbol{a}x + b$)
b	Y-intercept of the standard curve ($y = ax + \boldsymbol{b}$)
t	Time of the enzymatic reaction (20 mins)
F	The dilution factor of sample before assay
1000	1 mmol/L = 1000 µmol/L

Technical Support

For troubleshooting and technical assistance, please contact us at support@abbexa.com.