ab196989
Succinyl-CoA Synthetase Activity Assay Kit (Colorimetric)

Instructions for Use

For the rapid, sensitive and accurate measurement of Succinyl-CoA Synthetase activity in a variety of samples.

This product is for research use only and is not intended for diagnostic use.

PLEASE NOTE: With the acquisition of BioVision by Abcam, we have made some changes to component names and packaging to better align with our global standards as we work towards environmental-friendly and efficient growth. You are receiving the same high-quality products as always, with no changes to specifications or protocols.

Version 2a, Last Updated 23 August 2023

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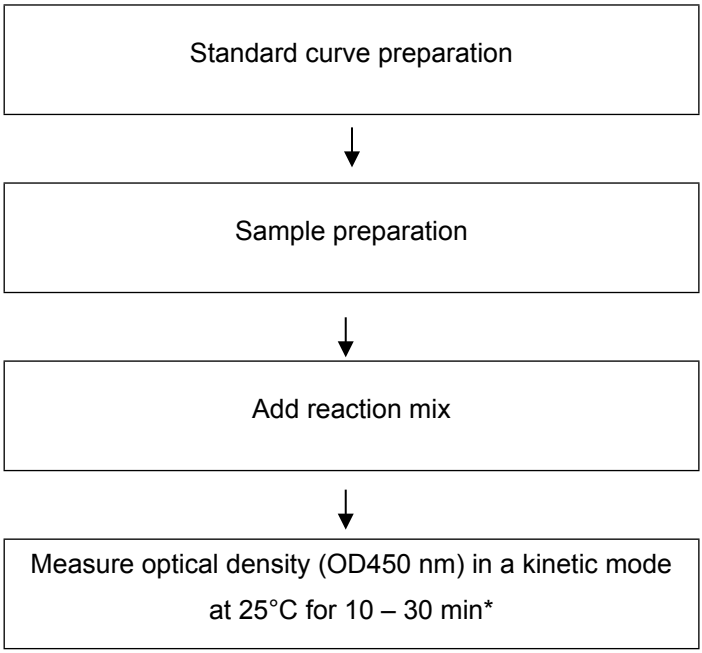
1. BACKGROUND

Succinyl-CoA Synthetase Activity Assay Kit (colorimetric) (ab196989) is an assay where Succinyl-CoA Synthetase (SCS) converts succinate into succinyl-CoA in the presence of ATP and CoA. Succinyl-CoA reacts with the Developer Solution III/SCS Developer to form a colored product with strong absorbance at 450 nm. This kit is simple to use and high throughput adaptable. It can detect less than 0.1 mU of succinyl-CoA synthetase activity in a variety of samples.

Succinyl-CoA Synthetase (SCS, also called Succinyl-CoA ligase, Succinate Thiokinase) 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.

2. ASSAY SUMMARY



**For kinetic mode detection, incubation time given in this summary is for guidance only.*

3. PRECAUTIONS

Please read these instructions carefully prior to beginning the assay.

All kit components have been formulated and quality control tested to function successfully as a kit. Modifications to the kit components or procedures may result in loss of performance.

4. STORAGE AND STABILITY

Store kit at -20ºC in the dark immediately upon receipt. Kit has a storage time of 1 year from receipt, providing components have not been reconstituted.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in section 5.

Aliquot components in working volumes before storing at the recommended temperature. **Reconstituted components are stable for 2 months.**

5. MATERIALS SUPPLIED

Item	Amount	Storage Condition (Before Preparation)	Storage Condition (After Preparation)
Assay Buffer VII/SCS Assay Buffer	25 mL	-20°C	-20°C
SCS Substrate Mix/SCS Substrate Mix (lyophilized)	1 vial	-20°C	-20°C
Enzyme Mix XI/SCS Enzyme Mix (lyophilized)	1 vial	-20°C	-20°C
Developer Solution III/SCS Developer (lyophilized)	1 vial	-20°C	-20°C
NADH Standard I/NADH Standard (lyophilized)	1 vial	-20°C	-20°C
SCS Positive Control/SCS Positive Control (lyophilized)	1 vial	-20°C	-80°C

6. MATERIALS REQUIRED, NOT SUPPLIED

These materials are not included in the kit, but will be required to successfully perform this assay:

- MilliQ water or other type of double distilled water (ddH_2O)
- PBS
- Microcentrifuge
- Pipettes and pipette tips
- Colorimetric microplate reader – equipped with filter for OD 450 nm
- 96 well plate: clear plates for colorimetric assay
- Heat block or water bath
- Dounce homogenizer or pestle (if using tissue)

For mitochondria isolation:

- Mitochondria Isolation Kit for Cultured Cells (ab110170), Mitochondria Isolation Kit for Cultured Cells (with Dounce Homogenizer) (ab110171), Mitochondria Isolation Kit for Tissue (ab110168) and Mitochondria Isolation Kit for Tissue (with Dounce Homogenizer) (ab110169)

7. LIMITATIONS

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- Do not use kit or components if it has exceeded the expiration date on the kit labels.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

8. TECHNICAL HINTS

- **This kit is sold based on number of tests. A 'test' simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.**
- Keep enzymes and heat labile components and samples on ice during the assay.
- Make sure all buffers and developing solutions are at room temperature before starting the experiment.
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions.
- Avoid foaming or bubbles when mixing or reconstituting components.
- Samples generating values higher than the highest standard should be further diluted in the appropriate sample dilution buffers.
- Ensure plates are properly sealed or covered during incubation steps.
- Ensure complete removal of all solutions and buffers from tubes or plates during wash steps.
- Make sure you have the appropriate type of plate for the detection method of choice.
- Make sure the heat block/water bath and microplate reader are switched on before starting the experiment.

9. REAGENT PREPARATION

- Briefly centrifuge small vials at low speed prior to opening.

9.1 **Assay Buffer VII/SCS Assay Buffer:**

Ready to use as supplied. Equilibrate to room temperature before use. Store at 4°C or -20°C.

9.2 **SCS Substrate Mix:**

Reconstitute in 220 µL ddH_2O. Gently pipette up and down to dissolve completely. Aliquot substrate so that you have enough volume to perform the desired number of tests. Store at -20°C. Use within 2 months. Keep on ice while in use.

9.3 **Enzyme Mix XI/SCS Enzyme Mix:**

Reconstitute in 220 µL Assay Buffer. Gently pipette up and down to dissolve completely. Aliquot enzyme mix so that you have enough volume to perform the desired number of tests. Store at -20°C. Use within 2 months. Keep on ice while in use.

9.4 **Developer Solution III/SCS Developer:**

Reconstitute in 220 µL ddH_2O. Aliquot Developer Solution III/developer so that you have enough volume to perform the desired number of tests. Store at -20°C. Use within 2 months. Keep on ice while in use.

9.5 **NADH Standard I/NADH Standard:**

Reconstitute in 400 µL ddH_2O to generate a 1.25 mM NADH Standard I/NADH standard solution. Aliquot standard so that you have enough volume to perform the desired number of tests. Store at -20°C. Use within 2 months. Keep on ice while in use.

9.6 **SCS Positive Control:**

Reconstitute in 100 µL of Assay Buffer and mix thoroughly. Aliquot positive control so that you have enough volume to perform the desired number of tests. Store at -80°C. Use within 2 months. Keep on ice while in use.

10. STANDARD PREPARATION

- Always prepare a fresh set of standards for every use.
- Diluted standard solution is unstable and must be used within 4 hours.

10.1 Using reconstituted 1.25 mM NADH Standard I/NADH Standard, prepare standard curve dilution as described in the table in a microplate or microcentrifuge tubes:

Standard #	Volume of Standard (µL)	Assay Buffer (µL)	Final volume standard in well (µL)	End Conc. NADH in well
1	0	150	50 µL	0 nmol/well
2	6	144	50 µL	2.5 nmol/well
3	8	142	50 µL	5.0 nmol/well
4	12	138	50 µL	7.5 nmol/well
5	16	134	50 µL	10 nmol/well
6	30	120	50 µL	12.5 nmol/well

Each dilution has enough amount of standard to set up duplicate readings (2 x 50 µL).

11. SAMPLE PREPARATION

General Sample information:

- We recommend performing several dilutions of your sample to ensure the readings are within the standard value range.
- We recommend that you use fresh samples. If you cannot perform the assay at the same time, we suggest that you complete the Sample Preparation step before storing the samples. Alternatively, if that is not possible, we suggest that you snap freeze cells or tissue in liquid nitrogen upon extraction and store the samples immediately at -80°C. When you are ready to test your samples, thaw them on ice. Be aware however that this might affect the stability of your samples and the readings can be lower than expected.

11.1 Cell (adherent or suspension) samples:

11.1.1 Harvest the amount of cells necessary for each assay (initial recommendation = 1 x 10^6 cells).

11.1.2 Wash cells with cold PBS.

11.1.3 Resuspend cells in 100 µL of ice cold Assay Buffer.

11.1.4 Homogenize cells quickly by pipetting up and down a few times. Keep on ice for 10 minutes.

11.1.5 Centrifuge sample for 5 minutes at 4°C at top speed using a cold microcentrifuge to remove any insoluble material.

11.1.6 Collect supernatant and transfer to a clean tube.

11.1.7 Keep on ice.

11.2 Tissue samples:

11.2.1 Harvest the amount of tissue necessary for each assay (initial recommendation = 10 mg).

11.2.2 Wash tissue in cold PBS.

11.2.3 Resuspend tissue in 100 µL of ice cold Assay Buffer.

11.2.4 Homogenize tissue with a Dounce homogenizer sitting on ice, with 10 – 15 passes. Keep on ice for 10 minutes.

11.2.5 Centrifuge samples for 5 minutes at 4°C at top speed using a cold microcentrifuge to remove any insoluble material.

11.2.6 Collect supernatant and transfer to a clean tube.

11.2.7 Keep on ice.

11.3 **Mitochondria:**

11.3.1 Isolate the mitochondria from fresh tissue or cells using a mitochondria isolation kit for tissue or cultured cells (see suggested products in Section 6).

NOTE: *We suggest using different volumes of sample to ensure readings are within the Standard Curve range.*

12. ASSAY PROCEDURE and DETECTION

- **Equilibrate all materials and prepared reagents to room temperature prior to use.**
- **It is recommended to assay all standards, controls and samples in duplicate.**
- **For samples containing succinate, set up background control sample well(s).**

12.1 **Set up Reaction wells:**

- Standard wells = 50 µL standard dilutions.
- Sample wells = 5 – 50 µL samples (adjust volume to 50 µL/well with Assay Buffer).
- Positive control wells = 1 – 10 µL SCS Positive Control (adjust volume to 50 µL/well with Assay Buffer).
- Background control sample wells= 1 – 50 µL samples (adjust volume to 50 µL/well with Assay Buffer.

12.2 **Reaction Mix:**

Prepare 50 µL of Reaction Mix for each reaction:

Component	SCS Reaction Mix (µL)	Background Control Mix (µL)
Assay Buffer VII/SCS Assay Buffer	**44**	**46**
SCS Substrate Mix	**2**	**0**
Enzyme Mix XI/SCS Enzyme Mix	**2**	**2**
Developer Solution III/SCS Developer	**2**	**2**

Mix enough reagents for the number of assays (samples, standards and positive control) to be performed. Prepare a master mix of the Reaction Mix to ensure consistency. We recommend the following calculation:

X µL component x (Number samples + Standards + positive control + 1).

12.3 Add 50 µL of Reaction Mix into each standard, sample, positive control wells. Mix well.

12.4 Add 50 µL of Background control mix into each background control sample well. Mix well.

12.5 Measure absorbance on a microplate reader at OD=450 nm in a kinetic mode, every 2 – 3 minutes, for 10 – 30 minutes at 25°C.

NOTE: *Sample incubation time can vary depending on the Succinyl-CoA Synthetase activity in samples. We recommend measuring the OD in kinetic mode, and choosing two time points (T_1 and 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).

13. CALCULATIONS

- Samples producing signals greater than that of the highest standard should be further diluted in appropriate buffer and reanalyzed, then multiplying the concentration found by the appropriate dilution factor.
- For statistical reasons, we recommend each sample should be assayed with a minimum of two replicates (duplicates).

13.1 Average the duplicate reading for each standard and sample.

13.2 If the sample background control is significant, then subtract the sample background control from sample reading.

13.3 Subtract the mean absorbance value of the blank (Standard #1) from all standard and sample readings. This is the corrected absorbance.

13.4 Plot the corrected absorbance values for each standard as a function of the final concentration of SCS.

13.5 Draw the best smooth curve through these points to construct the standard curve. Most plate reader software or Excel can plot these values and curve fit. Calculate the trendline equation based on your standard curve data (use the equation that provides the most accurate fit).

13.6 Extrapolate sample readings from the standard curve plotted using the following equation:

$$Time\ point\ value = \left(\frac{Corrected\ absorbance - (y - intercept)}{Slope}\right)$$

Time point = A1 or A2 (and ABG1 or ABG2 for the sample background control)

13.7 Activity of SCS is calculated as:

$\Delta OD450nm = (A_2 - A_{BG2}) - (A_1 - A_{BG1})$

13.8 Use the ΔOD to obtain B nmol of NADH generated.

13.9 Succinyl-CoA synthetase activity (in nmol/min/mL or mU/mL or U/mL) in the test samples is calculated as:

$$SCS\ Activity = \left(\frac{B}{((T2 - T1) \times V)}\right) * D$$

= nmol/min/mL = mU/mL = U/L

Where:

B = Amount of NADH from Standard Curve (nmol).

T1 = Time of the first reading (A_1) in minutes.

T2 = Time of the second reading (A_2) in minutes.

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

D = sample dilution factor.

Sample SCS Activity can also be expressed as mU/mg (nmoles/min NADH generated per mg of protein).

Unit Definition:

1 Unit SCS activity = amount of Succinyl-CoA-Synthetase that will generate 1.0 µmol of NADH per minute at pH7.4 at 25°C.

14. TYPICAL DATA

TYPICAL STANDARD CURVE – Data provided for **demonstration purposes only**. A new standard curve must be generated for each assay performed.

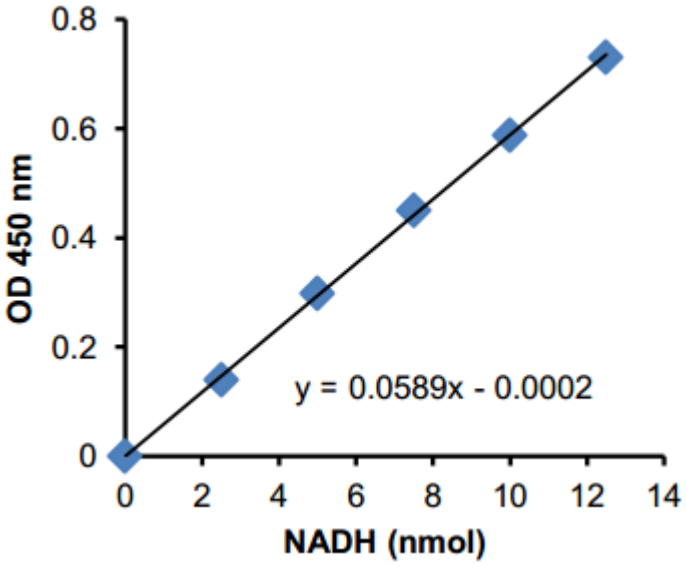


Figure 1. Typical NADH Standard calibration curve using colorimetric reading.

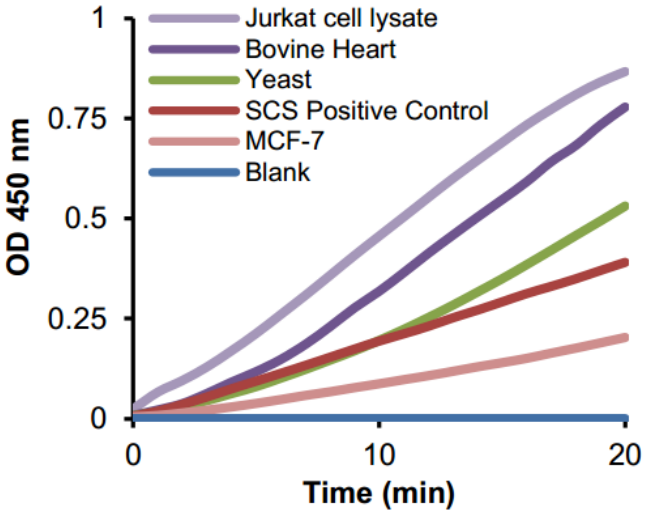


Figure 2: Succinyl-CoA Synthetase activity detected in mitochondria prepared from bovine heart, yeast (*P. pastoris*) and MCF-7 cells and in Jurkat cell lysate.

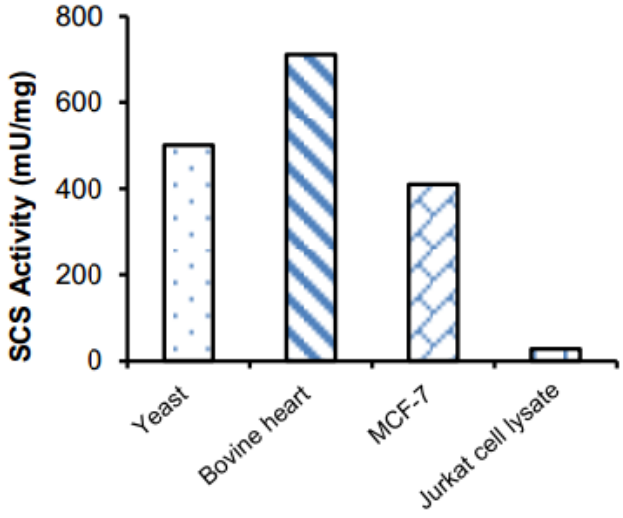


Figure 3: Succinyl-CoA Synthetase specific activity in mitochondria prepared from yeast (*P. pastoris*, 1.14 µg), bovine heart (1.1 µg) and MCF-7cells (0.5 µg), and in Jurkat cell lysate (25 µg).

15. QUICK ASSAY PROCEDURE

***NOTE**: This procedure is provided as a quick reference for experienced users. Follow the detailed procedure when performing the assay for the first time.*

- Prepare standard, substrate mix, Developer Solution III/developer, enzyme mix and positive control (aliquot if necessary); get equipment ready.
- Prepare standard curve.
- Prepare samples in duplicate (find optimal dilutions to fit standard curve readings).
- Set up plate for standard (50 µL), samples (50 µL) and background wells (50 µL) and positive control (50 µL).
- Prepare SCS Reaction Mix (Number samples + standards + positive control + 1); and Background control mix (Number background control samples + 1)

Component	Reaction Mix (µL)	Background Control Mix (µL)
Assay Buffer VII/SCS Assay Buffer	**44**	**46**
SCS Substrate Mix	**2**	**0**
Enzyme Mix XI/SCS Enzyme Mix	**2**	**2**
Developer Solution III/SCS Developer	**2**	**2**

- Add 50 µL of SCS Reaction Mix to the standard, sample and positive control wells.
- Add 50 µL of background control mix to the background control sample wells.
- Incubate plate at 25°C 10 – 30 minutes protected from light, and read absorbance at OD=450 nm in a kinetic mode.

16. TROUBLESHOOTING

Problem	Cause	Solution
Assay not working	Use of ice-cold buffer	Buffers must be at room temperature
	Plate read at incorrect wavelength	Check the wavelength and filter settings of instrument
	Use of a different 96-well plate	Colorimetric: Clear plates Fluorometric: black wells/clear bottom plate
Sample with erratic readings	Samples not deproteinized (if indicated on protocol)	Use PCA precipitation protocol for deproteinization
	Cells/tissue samples not homogenized completely	Use Dounce homogenizer, increase number of strokes
	Samples used after multiple free/ thaw cycles	Aliquot and freeze samples if needed to use multiple times
	Use of old or inappropriately stored samples	Use fresh samples or store at -80°C (after snap freeze in liquid nitrogen) till use
	Presence of interfering substance in the sample	Check protocol for interfering substances; deproteinize samples
Lower/ Higher readings in samples and Standards	Improperly thawed components	Thaw all components completely and mix gently before use
	Allowing reagents to sit for extended times on ice	Always thaw and prepare fresh reaction mix before use
	Incorrect incubation times or temperatures	Verify correct incubation times and temperatures in protocol

Problem	Cause	Solution
Standard readings do not follow a linear pattern	Pipetting errors in standard or reaction mix	Avoid pipetting small volumes (< 5 µL) and prepare a master mix whenever possible
	Air bubbles formed in well	Pipette gently against the wall of the tubes
	Standard stock is at incorrect concentration	Always refer to dilutions on protocol
Unanticipated results	Measured at incorrect wavelength	Check equipment and filter setting
	Samples contain interfering substances	Troubleshoot if it interferes with the kit
	Sample readings above/ below the linear range	Concentrate/ Dilute sample so it is within the linear range

17. **FAQ**

18. INTERFERENCES

These chemicals or biological materials will cause interferences in this assay causing compromised results or complete failure:

- RIPA: contains SDS which can destroy/decrease the activity of the enzyme.

19. **NOTES**

Technical Support

For all technical or commercial enquiries please go to: www.abcam.com/contactus

www.abcam.cn/contactus (China)

www.abcam.co.jp/contactus (Japan)