MA-0134

Succinate Assay

(100 wells, Colorimetric, OD 450 nm, Store at -20°C)

Background Information:

Succinate is an important metabolite in the (Krebs) tricarboxylic acid cycle. It lies at a key point providing $FADH_2$ and thus electrons entering the electron transport chain during cellular respiration. Succinate is formed from acetyl CoA and oxaloacetate by citrate synthase and is converted to fumarate with the formation of $FADH_2$. Succinate has also emerged as a signaling molecule in various cellular processes, including inflammation, hypoxia responses, and regulation of gene expression. In pathological conditions such as ischemia, it accumulates, impairing mitochondrial function and promoting oxidative stress and tissue damage. AkrivisBio's Succinate assay is a simple, sensitive method for the accurate determination of succinate at levels below 25 µM.

Assay Principle:

1 – Succinyl CoA synthetase in the presence of ATP and Coenzyme A converts succinate to succinyl CoA with the formation of ADP
2 – ADP is utilized by hexokinase to convert glucose to glucose-6-phosphate
3 – Glucose-6-phosphate is oxidized by glucose-6-phosphate dehydrogenase converting NAD to NADH
4 – NADH is used to reduce a tetrazolium to a highly colored formazan with a λ_{max} of 450 nm.

Assay Components:

Assay Buffer	25 ml	WM	MA-0134-A
Succinate-CoA Synthase	Lyoph	Purple	MA-0134-B
Hexokinase/G6P Dehydrogenase	Lyoph	Green	MA-0134-C
ATP/Coenzyme A/Glucose	Lyoph	Blue	MA-0134-D
NAD/WST8 Mix	Lyoph	Red	MA-0134-E
Succinate Standard	Lyoph	Yellow	MA-0134-F

Storage and Handling:

Store at -20°C prior to use. Warm Assay Buffer to room temperature before use. Centrifuge al small vials for a few seconds prior to opening.
Assay Buffer: Ready to use as supplied. Store at 4°C
Succinate-CoA Synthase, Hexokinase/G6P Dehydrogenase, ATP/Coenzyme A/Glucose: Reconstitute with 220 µl of Assay Buffer. If the assay is to be used multiple times over a period of time, aliquot into convenient portions and store at -20°C. Keep on ice while in use.
NAD/WST8 Mix: Reconstitute with 220 µl DI H_2O. Store at -20°C.
Succinate Standard: Reconstitute with 100 µl H_2O giving a 40 mM solution. Store at -20°C. Keep on ice while in use.

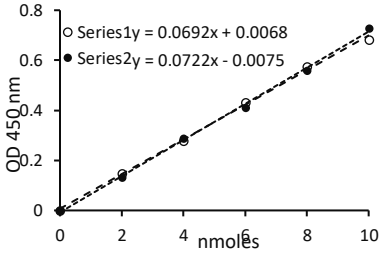
Assay Protocol:

1. Standard Curve: Transfer 10 µl of standard to 990 µl DI H_2O, giving a 0.4 mM solution. Transfer 0 – 5 – 10 – 15 – 20 – 25 µl of the standard solution to a series of wells in a 96-well plate giving 0, 2, 4, 6, 8, and 10 nmol of Succinate. Adjust all well volumes to 50 µl with Succinate Assay Buffer.
2. Sample Preparation: Homogenize tissue (10 mg) or cells (10^6) directly in 100 µl of assay buffer. Centrifuge at 16,000 X g to remove particulates. Transfer the clear supernatant to a fresh tube. Transfer 5-50 µl of each sample to a 96-well plate and bring all well volumes to 50 µl with Assay Buffer.
Notes: a. NADH in samples gives background. For samples with a significant NADH level, run samples in duplicate, using the paired well as a background control.
b. Decolorize colored samples such as wine using PVPP (1% w/v final concentration). Incubate for 5 minutes then filter through a 10 kDa spin filter.
3. Initiate Reaction: Prepare sufficient Reaction Mix for the number of samples and standards to be run. Each well requires 50 µl of Reaction Mix containing:

	Reaction Mix	* Background Control Mix
Assay Buffer	42 µl	44 µl
Succinate-CoA Synthase	2 µl	-----
Hexokinase/G6P Dehydrogenase	2 µl	2 µl
ATP/Coenzyme A/Glucose	2 µl	2 µl
NAD/WST8 Mix	2 µl	2 µl

Add 50 µl of the Reaction Mix to each well containing the Standards and samples, mix well.
4. Measurement: Monitor the reaction in a plate reader at 37°C for 30 minutes or longer at 450 nm
5. Typical Results:

nmole Standard	Standard Raw Values (OD)		Background Corrected Values (OD)	
0	0.1015	0.1033	0	0
2	0.2508	0.2355	0.1493	0.1321
4	0.3803	0.3914	0.2788	0.2880
6	0.5332	0.5144	0.4316	0.4111
8	0.6760	0.6645	0.5744	0.5611
10	0.7852	0.8326	0.6836	0.7292



6. Calculation: Subtract 0 Standard reading from all standard readings. Plot the Standard Curve and determine the slope of the standard curve. The slope defines the OD/nmol of the measurement system. If background controls have been run, subtract those values from the paired samples, otherwise, subtract the background value of the 0 standard. Divide the background corrected sample values by the slope of the standard curve to obtain nmoles of succinate in the sample wells. To convert back to nmoles of succinate per amount of sample:

A. Divide nmoles succinate in sample well by µl of sample applied to well = nmoles succinate/ µl sample
B. Multiply nmoles succinate/ µl sample X total volume of supernatant in fresh tube after centrifugation step = total nmoles succinate in sample.
C. Divide total nmoles succinate in sample by mg tissue (or # of cells, etc) = nmoles of succinate / mg tissue (or # of cells, etc.)

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