CREATIVE BIOMART®
Assay Kit

Succinate (Succinic Acid) Colorimetric Assay Kit

Product Information

Common Name

succinate

Cat.No. Kit-0807

Product Overview

Succinate (Succinic Acid) Assay Kit (Colorimetric) is used for measuring succinate using colorimetric methods.

Description

Succinic acid (C4H6O4) is distributed in all plants and animal tissues and was first obtained from amber. Due to its low toxicity, it is widely used in agriculture, food and pharmaceutical industry. Succinate (C4H4O4) is the salt or ester of succinic acid. It is one of the most active components of cellular respiratory and intracellular energy generation. Measurement of succinate or succinic acid level is a key to analysis of the citric acid cycle. Succinate (Succinic Acid) Assay Kit (Colorimetric) is a sensitive, fast and easy-to-use kit. In this assay, Succinate is utilized by Succinyl-CoA Synthetase to form an intermediate, which undergoes a series of reactions & reduces a colorless probe to a colored product with strong absorbance at 450 nm. This assay kit can detect less than 40 µM of succinate or succinic acid in a variety of sample types.

Applications

Measurement of succinate or succinic acid in various samples
Analysis of citric acid cycle in various tissues/cells

Storage

Store kit at –20°C, protect from light. Warm Assay Buffer to room temperature before use. Briefly centrifuge small vials prior to opening.
Read entire protocol before performing the experiment.

Kit Components

Succinate Assay Buffer 25 Ml

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Succinate Converter (Lyophilized) 1 vial
Succinate Enzyme Mix (Lyophilized) 1 vial
Succinate Substrate Mix (Lyophilized) 1 vial
Succinate Developer (Lyophilized) 1 vial
Succinate Standard (Lyophilized) 1 vial

Materials Required but Not Supplied

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

Compatible Sample Types

- Animal tissues such as liver, muscle, heart, spleen, lung etc.
- Cell culture: adherent or suspension cells
- Wine

Preparation

- Succinate Converter, Enzyme Mix and Substrate Mix: Reconstitute Succinate Converter, Enzyme Mix and Substrate Mix with 220 µl Succinate Assay Buffer. Pipette up and down to dissolve completely. Aliquot and store at -20°C. Avoid repeated freeze/thaw. Use within two months. Keep on ice while in use.
- Succinate Developer: Reconstitute with 220 µl dH2O. Pipette up and down to dissolve completely. Stable for 2 months at -20°C.
- Succinate Standard: Reconstitute with 100 µl dH2O to generate 100 mM (100 nmol/µl) Succinate Standard solution. Store at –20°C. Use within two months. Keep on ice while in use.

Assay Protocol

1. Sample Preparation: Tissue (10 mg) or cells (1 x 106) should be rapidly homogenized on ice with 100 µl of ice cold Succinate Assay Buffer. Centrifuge at 10,000 x g for 5 min. Collect the supernatant. Add 1-50 µl samples into a 96-well plate and bring the volume to 50 µl with Succinate Assay Buffer.
Notes:
a. For unknown samples, we suggest testing several doses of your samples to ensure the readings are within the Standard Curve range.

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b. NADH in samples will generate background. For samples having high NADH levels, prepare parallel sample well(s) as background control.
c. Succinate levels can be measured directly in liquid samples having slight color & ~ neutral pH. For colored liquid samples, we recommend to mix samples with polyvinylpyrrolidone (1% PVPP, w/v) and incubate for 5 min. at room temperature. Spin filter the samples by using 10 kDa spin column. For acidic samples such as white wine, neutralize the sample (1:1 dilution) with 0.5 M Tris HCl, pH 8.0.
2. Standard Curve Preparation: Dilute Succinate Standard to 1 mM (1 nmol/µl) by adding 10 µl of 100 mM Succinate Standard to 990 µl dH2O, mix well. Add 0, 2, 4, 6, 8 & 10 µl of diluted 1 mM Succinate Standard into a series of wells in a 96-well plate to generate 0, 2, 4, 6, 8, and 10 nmol/well of Succinate Standard. Adjust the volume to 50 µl/well with Succinate Assay Buffer.
3. Reaction Mix: Mix enough reagents for the number of assays (samples, background control and standards) to be performed. For each well, prepare 50 µl Reaction Mix containing:

	Reaction Mix *	Background Control Mix
Succinate Assay Buffer	42 µl	44 µl
Succinate Converter	2 µl	----
Succinate Enzyme Mix	2 µl	2 µl
Succinate Substrate Mix	2 µl	2 µl
Succinate Developer	2 µl	2 µl

Add 50 µl of the Reaction Mix to each well containing the Standards and samples, mix well.
* For samples having high NADH levels, add 50 µl of the Background Control Mix to sample background control well(s).
4. Measurement: Incubate the plate at 37°C for 30 min. Measure the absorbance at 450 nm.
5. Calculation: Subtract 0 Standard reading from all readings. Plot the Standard Curve. If the sample background control reading is significant, subtract the background control reading from samples readings. Apply the corrected sample reading to Succinate Standard Curve to get B nmol of Succinate in the sample wells.
Sample Succinate or Succinic Acid Concentration (C) = B/V x Dilution Factor = nmol/µl or µmol/ml or mM
Where: B is the amount of succinate or succinic acid from the Standard Curve (nmol)
V is the sample volume used in the reaction well (µl)

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Succinic Acid molecular weight: 118.09 g/mol.
Sample Succinate or Succinic Acid concentration can also be expressed in nmol/mg

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