Succinate Assay Kit

Succinate Assay Kit can be used to measure Succinate in food, beverage and other biological samples.

Catalog number: ARG82193

Package: 100 tests

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INTRODUCTION

Succinate (also known as Succinic acid) is a dicarboxylic acid with the chemical formula $(CH_2)_2(CO_2H)_2$. The name derives from Latin succinum, meaning amber. In living organisms, succinic acid takes the form of an anion, succinate, which has multiple biological roles as a metabolic intermediate being converted into fumarate by the enzyme succinate dehydrogenase in complex 2 of the electron transport chain which is involved in making ATP, and as a signaling molecule reflecting the cellular metabolic state. It is marketed as food additive E363. Succinate is generated in mitochondria via the tricarboxylic acid cycle (TCA). Succinate can exit the mitochondrial matrix and function in the cytoplasm as well as the extracellular space, changing gene expression patterns, modulating epigenetic landscape or demonstrating hormone-like signaling. As such, succinate links cellular metabolism, especially ATP formation, to the regulation of cellular function. Dysregulation of succinate synthesis, and therefore ATP synthesis, happens in some genetic mitochondrial diseases, such as Leigh syndrome, and Melas syndrome, and degradation can lead to pathological conditions, such as malignant transformation, inflammation and tissue injury.

As a food additive and dietary supplement, succinic acid is generally recognized as safe by the U.S. Food and Drug Administration. Succinic acid is used primarily as an acidity regulator in the food and beverage industry. It is also available as a flavoring agent, contributing a somewhat sour and astringent component to umami taste. As an excipient in pharmaceutical products, it is also used to control acidity or as a counter ion. Drugs involving succinate include metoprolol succinate, sumatriptan succinate, Doxylamine succinate or

solifenacin succinate. [Wikipedia: Succinate]

PRINCIPLE OF THE ASSAY

This Succinate Assay Kit provides a simple, and rapid procedure for measuring Succinate concentration in samples. In this assay, Succinate is converted to pyruvate. The pyruvate is then oxidized with the conversion of the dye in the kit into a colored and fluorescent form. The color intensity at 570 nm or fluorescence intensity at λ ex/em = 530/585 nm is directly proportional to the Succinate concentration in the sample. The concentration of Succinate in the sample is then determined by comparing the signals of samples to the standard.

MATERIALS PROVIDED & STORAGE INFORMATION

Component	Quantity	Storage information
Assay Buffer	10 ml (Ready to use)	-20°C
Succinate Standard (20 mM)	500 µl	-20°C
Enzyme Mixture	120 µl (Ready to use)	-20°C
Cosubstrate	120 µl (Ready to use)	-20°C
PEP	1 vial (Lyophilized)	-20°C
Dye Reagent	120 µl (Ready to use)	-20°C

The kit is shipped on ice. Store all components at -20°C in dark. Shelf life of six months after receipt.

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 570 nm. Or fluorescence Microplate Reader capable of measuring fluorescence at λ ex/em = 530/585 nm.
- Flat bottomed 96-well microplate or Black flat bottomed 96-well microplate
- Pipettes and pipette tips
- Deionized or distilled water.

TECHNICAL HINTS AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- The kit is shipped on ice. Store all components at -20°C in dark. Shelf life of six months after receipt.
- Before using the kit, spin tubes and bring down all components to the bottom of tubes.
- All materials should be equilibrated to room temperature (RT) before use.
- Ensure complete reconstitution and dilution of reagents prior to use.
- It is recommended that the standards and samples be assayed in at least duplicates.
- Change pipette tips between the addition of different reagent or samples.

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Collection:

Clear and slightly colored samples- Clear and slightly colored samples can be assayed directly. Sample might have to be diluted in distilled water before assay.

Solid samples- Solid samples such as food, fruits etc. can be homogenized in water followed by filtration or centrifuge at 14,000 rpm for 5 min.

All samples can be stored at –80 to –20°C for at least one month.

Dilution:

- For Soy Sauce and Red Wine. Sample has to be diluted 1:30 to 1:50 in distilled water for colorimetric analysis, or 1:300 to 1:500 for fluorimetric analysis.
- If the initial assay found samples contain Succinate higher than 400 µM for the colorimetric assay, or 40 µM for the fluorimetric assay, the samples can be diluted with distilled water and then re-assay the samples. For the calculation of the concentrations this dilution factor (n) has to be taken into account. The sample must be well mixed with the diluents buffer before assay.

(It is recommended to do pre-test to determine the suitable dilution factor).

REAGENT PREPARATION

- **PEP:** Dissolve the PEP in 120 µl of distilled water. Pipette up and down to mix well, and make sure the PEP is fully dissolved completely before assay. The reconstituted PEP is stable for 4 weeks when it stored at -20°C. Before each use of the PEP, pipette up and down or brief mix to assure the enzyme is mixed well.
- **Standard:**

  For Colorimetric Procedure:

  - Internal standard: Mixing 20 µL of 20 mM Standard stock and 380 µL deionized water to yield 400 µL of 1 mM Succinate Internal standard. 5 µL of 1 mM Succinate is used for each internal standard test.

  For fluorimetric Procedure:

  - For fluorimetric assays, the linear detection range is 2 to 40 µM Succinate.
  - Mixing 20 µL of 20 mM Standard stock and 380 µL of deionized water to yield 400 µL of 1 mM Succinate.
  - Dilute the 1 mM Succinate standard with deionized water to yield standard concentration as 40 µM, 24 µM and 12 µM.

Standard No.	Standard Conc. µM	Deionized water (µl)	Standard (µl)
S1	40	480	20 µl of 1 mM Stock
S2	24	40	60 µl of S1
S3	12	70	30 µl of S1
S0	0	100	0

- **Working Reagent:**

  For each reaction combine the following (*Prepare before use)*:

  85 µL of Assay Buffer

  1 µL of Enzyme Mixture

  1 µL of Cosubstrate

  1 µL of PEP

  1 µL Dye Reagent.

  Transfer 80 µl of Working Reagent to each sample and standard wells.

ASSAY PROCEDURE

All materials should be equilibrated to room temperature (RT) before use, each vial should be mixed thoroughly without foaming and briefly centrifuge tubes prior to use. Each sample requires a sample blank.

For Colorimetric Procedure:

1. Add 20 µl of each sample in two separate wells in flat bottomed 96 well plate.

   Well 1: sample alone for sample assay:

   Add 20 µL of sample and 5 µL of dH2O.

   Well 2: sample plus internal standard:

   Add 20 µL of sample and 5 µL of 1 mM Succinate (internal standard).
2. Add 20 +5 µl of dH2O in separate wells in flat bottomed 96 well plate as Blank.
3. Add 80 µL of the Working Reagent to each well.

4. Gently tap the plate to ensure thorough mixing. Incubate for 30 min at room temperature in dark.
5. Read the OD with a microplate reader at 570 nm (550 - 585nm) immediately.

For Fluorimetric Procedure (black 96 well plate is used):

1. Add 20 µL of samples and each standard (S0-S3) to separate wells in the black 96 well plate.
2. Add 80 µL of the Working Reagent to each well.
3. Gently tap the plate to ensure thorough mixing. Incubate for 30 min at room temperature in dark.
4. Read fluorescence intensity at λ ex = 530 nm and λ em = 585 nm immediately.

Summary:

A. For Colorimetric Procedure:

	Assayed sample	Samples plus Internal Standard	Blank
Sample	20 µl	20 µl	-
1 mM Standard	-	5 µl	-
dH2O	5 µl	-	25 µl
Working Reagent	80 µl	80 µl	80 µl
Mix well and incubate for 30 min at RT in dark.			
Read the OD with a microplate reader at 570nm immediately.			

Note: The concentration of Standard: 1 mM Succinate. Please refer the detail at REAGENT PREPARATION section.

B. For Fluorimetric Procedure:

	Assayed sample	Standard S0-S3
Sample	20 µl	-
Standards	-	20 µl
Working Reagent	80 µl	80 µl
Mix well and incubate for 30 min at RT in dark.		
Read fluorescence intensity at λ ex/em = 530 / 585 nm immediately.)		

Note: The concentration of Standard: 40 µM, 24 µM and 12 µM Succinate. Please refer the detail at REAGENT PREPARATION section.

CALCULATION OF RESULTS

1. For Colorimetric Method: the sample Succinate concentration is computed as follows:

$$[\text{Succinate}]\ (\mu M) = N \times [(OD_{\text{Sample}} - OD_{\text{Blank}}) / (OD_{\text{Standard}} - OD_{\text{Sample}})] \times (\text{Standard Conc.} / 4]$$

$$= N \times 250 \times [(OD_{\text{Sample}} - OD_{\text{Blank}}) / (OD_{\text{Standard}} - OD_{\text{Sample}})]$$

Note:

OD_{Sample} : OD value of Assayed sample well

OD_{Standard} : OD value of Samples plus Internal Standard

OD_{Blank} : OD value of Blank well

N = dilution factor (if sample has been diluted before assay)

2. For Fluorimetric Method: Subtract the blank value (S0) from the standard values and plot the ΔF against standard concentrations. Determine the slope and calculate the Succinate concentration of the Samples as follows:

   [Succinate] (µM)=

   N X [(F_{Sample} – F_{Blank}) / Slope (μM^{-1})]

   Note:

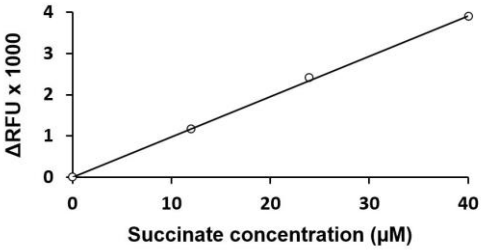
   F_{Sample} : Fluorescence value of Assayed sample well

   F_{Blank} : Fluorescence value of Blank well (S0)

   N = dilution factor (if sample has been diluted before assay)

3. If the calculated Succinate concentration is >400 µM for the colorimetric assay, or >40 µM for the fluorimetric assay, dilute sample in deionized water and repeat assay. Multiply result by the dilution factor N.
4. Conversions: 1 mM succinate equals 11.7 mg/dL, or 117 ppm.

EXAMPLE OF ASSAY



QUALITY ASSURANCE

Sensitivity

Linear detection range:

Colorimetric assays: 10 to 400 µM

Fluorimetric assays: 2 to 40 µM

The minimum detectable dose (MDD) of Succinate was:

Colorimetric assays: 10 µM

Fluorimetric assays: 2 µM