Succinate Assay Kit (Colorimetric/Fluorometric)

LS-K166-100 (100 Tests) • **Store at -20°C**

Introduction

SUCCINATE, or succinic acid, can be found in all plants and animal tissues. It is an intermediate in the citric acid cycle and plays an important role in intracellular energy generation. Succinate is widely used as a flavoring agent in the food, beverage, and pharmaceutical industries due to its low toxicity.

This succinate assay provides a simple, one step assay for measuring succinate. In this assay succinate is converted to pyruvate which reacts with specific reagents and dye to form a colored product. The color intensity at 570 nm or fluorescence at $\lambda_{ex/em}$ = 530/585 nm of the reaction product is directly proportional to succinate concentration in the sample.

Key Features

- Fast and sensitive. Use 20 µL sample. Linear detection range 10 to 400 µM for colorimetric assays and 2 to 40 µM for fluorometric assays.
- Convenient. The procedure involves adding a single working reagent, and reading the absorbance or fluorescence after 30 minutes. Room temperature assay. No 37°C heater is needed.
- High-throughput. Can be readily automated as a high-throughput 96-well plate assay for thousands of samples per day.

Applications

- Direct Assays: succinate in food, beverage, agricultural products, and other biological samples.

Components

Component	K166-100
	100 Tests
Assay Buffer	10 mL
Enzyme Mix	120 µL
Cosubstrate	120 µL
PEP	Dried
Dye Reagent	120 µL
Standard (20 mM Succinate)	500 µL

Materials Not Supplied

Pipetting devices, centrifuge tubes, clear flat-bottom 96-well plates (e.g. VWR cat# 82050-760), and plate or cuvette reader.

Storage

The kit is shipped on ice. Store all components at -20°C upon receiving. Shelf life: 6 months after receipt.

LifeSpan BioSciences, Inc. • 2401 Fourth Avenue, Suite 900, Seattle, WA 98121
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Assay Procedure

Reagent Preparation

Reconstitute PEP by adding 120 µL water to tube. Make sure PEP is fully dissolved by pipetting up and down. Store reconstituted PEP at -20°C and use within 1 month.

Sample Preparation

Clear and slightly colored samples can be assayed directly. It is prudent to test several dilutions to determine an optimal dilution factor *n*.

Solid samples (food, fruits, etc.) can be homogenized in water followed by filtration or centrifugation (e.g. 5 min 14,000 rpm).

Samples Tested: Soy Sauce and Red Wine. Each diluted 1:30 to 1:50 in dH_2O for colorimetric analysis, or 1:300 to 1:500 for fluorometric analysis.

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

Colorimetric Procedure

1. Internal standard is required for colorimetric assay. Each sample requires two separate reactions: 1) Sample plus internal standard and 2) Sample alone. In addition, each assay plate requires a water blank well. Add 20 µL of each sample to two separate wells. Also, add 20 µL dH_2O to a separate well. For the internal standard, prepare 400 µL 1 mM succinate standard by mixing 20 µL 20 mM standard with 380 µL dH_2O. Add 5 µL 1 mM standard to the sample plus internal standard wells. Add 5 µL dH_2O to the sample alone and water wells.
2. Prepare sufficient Working Reagent (WR) for wells by mixing, for each well, 85 µL Assay Buffer, 1 µL Enzyme Mix, 1 µL Cosubstrate, 1 µL PEP and 1 µL dye reagent. Fresh reconstitution of the WR is recommended. Add 80 µL WR to each well. Tap plate to mix. Incubate for 30 min at room temperature.
3. Read optical density at 570nm (550-585nm).

Fluorometric Procedure

1. Prepare a 40 µM Standard Premix by mixing 20 µL of 1 mM succinate (see colorimetric internal standard procedure) with 480 µL dH_2O. Dilute Standard in distilled water as follows.

No	Premix + H_2O	Vol (µL)	Succinate (µM)
1	100 µL + 0 µL	100	40
2	60 µL + 40 µL	100	24
3	30 µL + 70 µL	100	12
4	0 µL + 100 µL	100	0

Transfer 20 µL standards and 20 µL samples into separate wells of a black 96-well plate.

2. Add 80 µL Working Reagent (see Colorimetric Procedure). Tap plate to mix.
3. Incubate 30 min at room temperature and read fluorescence at $\lambda_{ex/em}$ = 530/585 nm.

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Calculations

Colorimetric Method: the succinate concentration is computed as follows:

$$[\text{Succinate}] = \frac{R_{SAMPLE} - R_{H_2O}}{R_{STANDARD} - R_{SAMPLE}} \times 250 \times n \quad (\mu M)$$

where R_{SAMPLE}, R_{H2O}, and $R_{STANDARD}$ are optical density of the Sample, Water, and the Sample plus Standard, respectively. *n* is the sample dilution factor. Note: The volume of the internal standard is 4× lower than the sample volume; thus, the sample to standard ratio is multiplied by 250 µM and not 1000 µM.

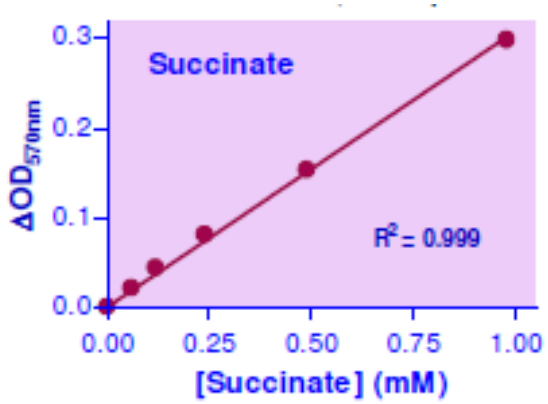
Fluorometric Method: Determine the Slope from the standard fluorescence values and calculate the succinate concentration as follows:

$$[\text{Succinate}] = \frac{R_{SAMPLE} - R_{H_2O}}{\text{Slope } (\mu M^{-1})} \times n \quad (\mu M)$$

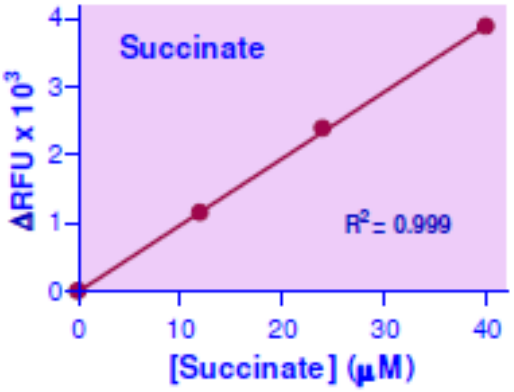
Notes: If the calculated succinate concentration is >400 µM for the colorimetric assay, or >40 µM for the fluorometric assay, dilute sample in dH_2O and repeat assay. Multiply result by the dilution factor *n*.

Conversions: 1 mM succinate equals 11.7 mg/dL, or 117 ppm.

Sample Data



96-well Colorimetric Assay: Internal Standard Linearity in Red Wine



96-well Fluorometric Assay

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