EnzyChrom™ Succinate Assay Kit (ESNT-100)

Quantitative Colorimetric/Fluorimetric Succinate (Succinic Acid) Determination

DESCRIPTION

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.

BioAssay Systems' 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 of 20 µL sample. Linear detection range 10 µM to 400 µM for colorimetric assays and 2 to 40 µM for fluorimetric 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.

KIT CONTENTS (100 TESTS IN 96-WELL PLATES)

Assay Buffer:	10 mL	**Enzyme Mix:**	120 µL
Cosubstrate:	120 µL	**PEP:**	Dried
Dye Reagent:	120 µL	**Standard:**	500 µL 20 mM Succinate

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

Precautions: reagents are for research use only. Briefly centrifuge tubes before opening. Equilibrate all components to room temperature prior assay. Normal precautions for laboratory reagents should be exercised while using the reagents. Please refer to Material Safety Data Sheet for detailed information.

PROCEDURES

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 fluorimetric 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)

Fluorimetric 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.

CALCULATION

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.

Fluorimetric Method: Determine the Slope from the standard fluorescence values and calculate the succcinate concentration as follows:

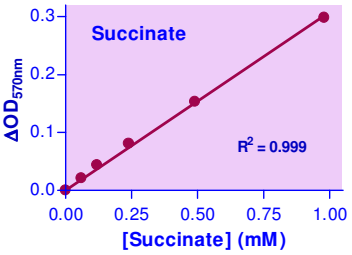
$$[\text{Succinate}] = \frac{R_{SAMPLE} - R_{H_2O}}{\text{Slope } (\mu M^{-1})} \times n \quad (\mu M)$$

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

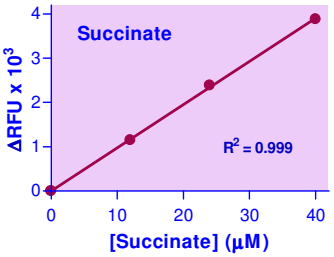
Conversions: 1 mM succinate equals 11.7 mg/dL, or 117 ppm.

MATERIALS REQUIRED, BUT NOT PROVIDED

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



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



96-well Fluorimetric Assay

LITERATURE

1. Mills, E. and O'Neill L. (2014). Succinate: A Metabolic Signal in Inflammation. Trends in Cell Biology 24(5), 313-320.
2. Li, X. et al. (2013). Identification of the Kinetic Mechanism of Succinyl-CoA Synthetase. BioSci Rep. 33(1):145-63.
3. Thakker C, et al (2012). Succinate production in Escherichia coli. Biotechnol J. 7(2):213-24.