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Product Information

Succinate Assay Kit

Catalog Number **MAK335**
Storage Temperature –20 °C

TECHNICAL BULLETIN

Product Description
Succinate, or succinic acid, can be found in all plants and animal tissues. It is an intermediate in the citric acid (TCA) 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.

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

The Succinic Assay Kit is suitable for succinate determination in food, beverage, agricultural products, and other biological samples. Linear detection range is 10–400 μM for colorimetric assays and 2–40 μM for fluorimetric assays.

Components
The kit is sufficient for 100 colorimetric or fluorometric assays in 96 well plates.

Assay Buffer Catalog Number MAK335A	10 mL
Enzyme Mix Catalog Number MAK335B	120 μL
Cosubstrate Catalog Number MAK335C	120 μL
PEP Catalog Number MAK335D	1 Vial
Dye Reagent Catalog Number MAK335E	120 μL
Standard (20 mM Succinate) Catalog Number MAK335F	500 μL

Reagents and Equipment Required but Not Provided.
- Pipetting devices and accessories (e.g., multichannel pipettor)
- Centrifuge tubes
- 96 well flat bottom plate – It is recommended to use black plates with clear bottoms for fluorescence assays and clear plates for colorimetric assays.
- Fluorescence or spectrophotometric multiwell plate reader

Precautions and Disclaimer
This product is for R&D use only, not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability
The kit is shipped on dry ice. Store all components at –20 °C upon receiving.

Preparation Instructions
Reagent Preparation
Briefly centrifuge tubes before opening. Equilibrate all components to room temperature prior to assay.

Reconstitute PEP by adding 120 μL of ultrapure water to vial. 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 ultrapure water followed by filtration or centrifugation (e.g., 5 minutes at 14,000 rpm).

Examples of samples tested are soy sauce and red wine. Each sample was diluted 1:30 to 1:50 in ultrapure water 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.

Procedures
Colorimetric Procedure
Note: An internal standard is required for the colorimetric assay. Each sample requires two separate reactions:

1. Sample plus internal standard
2. Sample alone

In addition, each assay plate requires a water blank well.

Standard Preparation – Prepare 400 µL of 1 mM Succinate Standard by mixing 20 µL of 20 mM standard with 380 µL of ultrapure water.

Reagent Mix Preparation – For each well of reaction, prepare Reagent Mix by mixing into a clean tube:
85 µL Assay Buffer
1 µL Enzyme Mix
1 µL Cosubstrate
1 µL PEP Solution (See Reagent Preparation)
1 µL Dye Reagent

Fresh reconstitution of the Reagent Mix is recommended

1. Add 20 µL of each sample to two separate wells. Also, add 20 µL of ultrapure water to a separate well.
2. Add 5 µL of 1 mM standard to the sample plus internal standard wells.
3. Add 5 µL of ultrapure water to the sample alone and water wells.
4. Add 80 µL of Working Reagent to each well. Tap plate to mix.
5. Incubate for 30 minutes at room temperature.
6. Measure the absorbance at 570 nm (A_{570}).

Fluorometric Procedure
Standards Preparation – Prepare a 40 µM Standard Premix by mixing 20 µL of 1 mM Succinate Standard (see Colorimetric Standard Preparation procedure) with 480 µL of ultrapure water. Prepare 4 standards by dilution of 40 µM Standard Premix with ultrapure water (see Table 1).

Table 1.
Preparation of Succinate Standards

Tube	Standard Premix	Ultrapure Water	Succinate (µM)
1	100 µL	0 µL	40
2	60 µL	40 µL	24
3	30 µL	70 µL	12
4	0 µL	100 µL	0

1. Transfer 20 µL of standards and 20 µL of samples into separate wells of a black 96 well plate.
2. Add 80 µL of Reagent Mix (see Colorimetric Reagent Mix Preparation procedure).
3. Tap plate to mix. Incubate 30 minutes at room temperature.
4. Read fluorescence at λ_{ex} = 530 nm/λ_{em} = 585 nm.

Results

Colorimetric Procedure

The succinate concentration is calculated as follows:

$$\text{Succinate } (\mu M) = \frac{(A_{570})_{sample} - (A_{570})_{water\ blank}}{(A_{570})_{standard} - (A_{570})_{sample}} \times 250 \times n$$

$(A_{570})_{sample}$, $(A_{570})_{water\ blank}$, and $(A_{570})_{standard}$ = the optical density of the sample, water blank, and standard, respectively.

250 = The volume of the internal standard is 4× less than the sample volume; thus, the sample to standard ratio is multiplied by 250 μM and not 1,000 μM.

n = sample dilution factor

Fluorometric

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

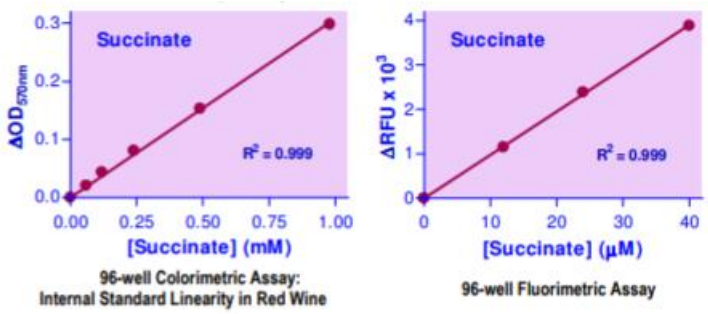
$$\text{Succinate } (\mu M) = \frac{F_{sample} - F_{water\ blank}}{\text{Slope } (\mu M^{-1})} \times n$$

F_{sample} and $F_{water\ blank}$ = the fluorescence of the sample and water blank, respectively.

n = sample dilution factor

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

Figure 1.



Conversions

1 mM succinate equals 11.7 mg/dL or 117 ppm

References

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

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