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# Product Information

**Succinate Colorimetric Assay Kit**
(Succinic Acid)

Catalog Number **MAK184**
Storage Temperature –20 °C

## TECHNICAL BULLETIN

### Product Description

Succinate (succinic acid) is a citric acid cycle intermediate and functions in the transport of electrons to the mitochondrial electron transport chain. It also functions in innate immune signaling and induces IL-1β production during inflammation.[1] Succinates have been often used for the treatment of hypertension.[2] Succinate measurements can be useful for studying its metabolic, immunological, and therapeutic activities.

The Succinate Colorimetric Assay Kit is a highly sensitive assay for determining succinate levels (ranging from 2–10 nmole/well) in a variety of samples. Succinate concentration is determined by a coupled enzyme reaction, which results in a colorimetric (450 nm) product proportional to the succinate present.

### Components

The kit is sufficient for 100 assays in 96 well plates.

| | |
|---|---|
| Succinate Assay Buffer<br>Catalog Number MAK184A | 25 mL |
| Succinate Converter<br>Catalog Number MAK184B | 1 vl |
| Succinate Enzyme Mix<br>Catalog Number MAK184C | 1 vl |
| Succinate Substrate Mix<br>Catalog Number MAK184D | 1 vl |
| Succinate Developer<br>Catalog Number MAK184E | 1 vl |
| Succinate Standard<br>Catalog Number MAK184F | 1 vl |

### Reagents and Equipment Required but Not Provided.

- 96 well flat-bottom plate – It is recommended to use clear plates for colorimetric assays.
- Spectrophotometric multiwell plate reader
- Polyvinylpyrrolidine (PVPP), optional for colored liquid samples
- 10 kDa Molecular Weight Cut-Off (MWCO) spin filter, optional for colored liquid samples
- Tris HCl, pH 8.0, optional for acidic samples

### 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.

### Preparation Instructions

Briefly centrifuge vials before opening. Use ultrapure water for the preparation of reagents. To maintain reagent integrity, avoid repeated freeze/thaw cycles.

Succinate Assay Buffer – Allow buffer to come to room temperature before use.

Succinate Converter, Enzyme Mix, and Substrate Mix – Reconstitute each with 220 µL of Succinate Assay Buffer. Mix well by pipetting, then aliquot and store, protected from light, at –20 °C. Keep on ice while in use. Use within 2 months of reconstitution.

Succinate Developer – Reconstitute with 220 µL of water. Mix well by pipetting, then aliquot and store, protected from light, at –20 °C.

Succinate Standard – Reconstitute with 100 µL of water to generate 100 mM (100 nmole/µL) Succinate Standard solution. Mix well by pipetting, then aliquot and store, protected from light, at –20 °C. Keep on ice while in use. Use within 2 months of reconstitution.

**Storage/Stability**
The kit is shipped on wet ice. Storage at –20 °C, protected from light, is recommended.

**Procedure**
All samples and standards should be run in duplicate.

Succinate Standards for Colorimetric Detection
Dilute 10 µL of the 100 mM (100 nmole/µL) Succinate Standard Solution with 990 µL of water to prepare a 1 mM (1 nmole/µL) standard solution. Add 0, 2, 4, 6, 8, and 10 µL of the 1 mM Succinate standard solution into a 96 well plate, generating 0 (blank), 2, 4, 6, 8, and 10 nmole/well standards. Add Succinate Assay Buffer to each well to bring the volume to 50 µL.

Sample Preparation
Tissue (10 mg) or cells ($1 \times 10^6$) should be rapidly homogenized on ice in 100 µL of ice-cold Succinate Assay Buffer. Centrifuge at 10,000 × *g* for 5 minutes to remove insoluble material. Collect the supernatant.

Liquid samples with slight color and neutral pH can be directly added to the wells.

Significant color in samples may impact the assay. To reduce color impact, mix samples with 1% polyvinylpyrrolidone (PVPP) and incubate for 5 minutes at room temperature. Centrifuge with a 10 kDa MWCO spin filter.

Acidic samples may also impact the assay. To neutralize the sample, dilute 1:1 with 0.5 M Tris HCl, pH 8.0.

Add 1–50 µL samples into duplicate wells of a 96 well plate. Bring samples to a final volume of 50 µL with Succinate Assay Buffer.

For unknown samples, it is suggested to test several sample dilutions to ensure the readings are within the linear range of the standard curve.

High levels of NADH can result in sample background. To correct for the background, include a Sample Blank for each sample by omitting the Succinate Converter. The Sample Blank readings can then be subtracted from the sample readings.

Assay Reaction
1. Set up the Reaction Mixes according to the scheme in Table 1. 50 µL of the appropriate Reaction Mix is required for each reaction (well).

**Table 1.**
Reaction Mixes

| Reagent | Samples and Standards | Sample Blank |
|---|---|---|
| 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 |

2. Add 50 µL of the appropriate Reaction Mix to each of the wells. Mix well using a horizontal shaker or by pipetting, and incubate the reaction for 30 minutes at 37 °C. Protect the plate from light during the incubation.

3. Measure the absorbance at 450 nm ($A_{450}$).

**Results**

Calculations

The background for the assays is the value obtained for the 0 (blank) Succinate Standard. Correct for the background by subtracting the blank value from all readings. Background values can be significant and must be subtracted from all readings. Use the values obtained from the appropriate Succinate Standards to plot a standard curve.

Note: A new standard curve must be set up each time the assay is run.

Subtract the Sample Blank value from the sample reading to obtain the corrected measurement. Using the corrected measurement, the amount of Succinate present in the sample may be determined from the standard curve.

Concentration of Succinate

$S_a/S_v = C$

where:

$S_a$ = Amount of Succinate in sample well (nmole) from standard curve

$S_v$ = Sample volume (µL) added into the well

C = Concentration of Succinate in sample

Succinate molecular weight: 118.09 g/mole

Sample Calculation

Amount of Succinate ($S_a$) = 4.84 nmole (from standard curve)

Sample volume ($S_v$) = 50 µL

Concentration of Succinate in sample:

$$4.84 \text{ nmole}/50 \ \mu\text{L} = 0.0968 \text{ nmole}/\mu\text{L}$$

$$0.0968 \text{ nmole}/\mu\text{L} \times 118.09 \text{ ng/nmole} = 11.43 \text{ ng}/\mu\text{L}$$

**References**


1. Tannahill, G.M. et al., Succinate is an inflammatory signal that induces IL-1β through HIF-1α. Nature, **496(7444)**, 238–242 (2013).
2. Heffernan, K.S. et al., Effect of atenolol vs metoprolol succinate on vascular function in patients with hypertension. Clin. Cardiol., **34(1)**, 39–44 (2011).

**Troubleshooting Guide**

| Problem | Possible Cause | Suggested Solution |
|---|---|---|
| Assay not working | Cold Assay Buffer | Assay Buffer must be at room temperature |
| | Omission of step in procedure | Refer and follow Technical Bulletin precisely |
| | Plate reader at incorrect wavelength | Check filter settings of instrument |
| | Type of 96 well plate used | For colorimetric assays, use clear plates |
| Samples with erratic readings | Samples prepared in different buffer | Use the Assay Buffer provided or refer to Technical Bulletin for instructions |
| | Cell/Tissue culture samples were incompletely homogenized | Repeat the sample homogenization, increasing the length and extent of homogenization step. |
| | Samples used after multiple freeze-thaw cycles | Aliquot and freeze samples if samples will be used multiple times |
| | Presence of interfering substance in the sample | If possible, dilute sample further |
| | Use of old or inappropriately stored samples | Use fresh samples and store correctly until use |
| Lower/higher readings in samples and standards | Improperly thawed components | Thaw all components completely and mix gently before use |
| | Use of expired kit or improperly stored reagents | Check the expiration date and store the components appropriately |
| | Allowing the reagents to sit for extended times on ice | Prepare fresh Master Reaction Mix before each use |
| | Incorrect incubation times or temperatures | Refer to Technical Bulletin and verify correct incubation times and temperatures |
| | Incorrect volumes used | Use calibrated pipettes and aliquot correctly |
| Non-linear standard curve | Use of partially thawed components | Thaw and resuspend all components before preparing the reaction mix |
| | Pipetting errors in preparation of standards | Avoid pipetting small volumes |
| | Pipetting errors in the Reaction Mix | Prepare a Master Reaction Mix whenever possible |
| | Air bubbles formed in well | Pipette gently against the wall of the tubes |
| | Standard stock is at incorrect concentration | Refer to the standard dilution instructions in the Technical Bulletin |
| | Calculation errors | Recheck calculations after referring to Technical Bulletin |
| | Substituting reagents from older kits/lots | Use fresh components from the same kit |
| Unanticipated results | Samples measured at incorrect wavelength | Check the equipment and filter settings |
| | Samples contain interfering substances | If possible, dilute sample further |
| | Sample readings above/below the linear range | Concentrate or dilute samples so readings are in the linear range |

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