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# Product Information

**Succinate Dehydrogenase Activity Colorimetric Assay Kit**

Catalog Number **MAK197**
Storage Temperature –20 °C

## TECHNICAL BULLETIN

### Product Description

Succinate dehydrogenase (SDH; EC 1.3.5.1) is a mitochondrial enzyme that catalyzes the oxidation of succinate to fumarate and carries electrons from FADH to CoQ in eukaryotes and bacteria. It has a central function in the maintenance of cellular energy metabolism via the Krebs (tricarboxylic acid) cycle and the electron transport chain.[1,2] Mutations in SDH cause hereditary paraganglioma/phaeochromocytoma syndrome and a neurodegenerative disorder known as Leigh syndrome.[2,3]

The Succinate Dehydrogenase Activity Colorimetric Assay kit provides a simple and sensitive procedure for measuring SDH activity in a variety of tissues, cells, and isolated mitochondria. SDH activity is determined by generating a product with absorbance at 600 nm proportional to the enzymatic activity present. One unit of SDH is the amount of enzyme that generates 1.0 μmole of DCIP per minute at pH 7.2 at 25 °C.

### Components

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

| | |
|---|---|
| SDH Assay Buffer<br>Catalog Number MAK197A | 25 mL |
| SDH Substrate Mix<br>Catalog Number MAK197B | 1 vl |
| SDH Probe<br>Catalog Number MAK197C | 0.2 mL |
| DCIP Standard, 2 mM<br>Catalog Number MAK197D | 0.4 mL |
| SDH Positive Control<br>Catalog Number MAK197E | 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
- Mitochondria Isolation Kit (optional for mitochondria samples, Catalog Number MITOISO1 for tissue, MITOISO2 for cells, MITOISO3 for yeast, or equivalent)

### Precautions and Disclaimer

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.

SDH Assay Buffer – Store the buffer at –20 °C or 2–8 °C. Allow buffer to come to room temperature before use.

SDH Substrate Mix – Reconstitute with 220 μL of water. Mix well by pipetting. Aliquot and store at –20 °C. Keep on ice during use.

SDH Probe and DCIP Standard – Store at –20 °C. Allow to come to room temperature before use.

SDH Positive Control – Reconstitute with 100 μL of SDH Assay Buffer. Mix well by pipetting. Aliquot and store at –80 °C. Keep on ice during use.

### Storage/Stability

The kit is shipped on wet ice and storage at –20 °C, protected from light, is recommended.

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

DCIP Standards for Colorimetric Detection
Add 0, 4, 8, 12, 16, and 20 µL of the 2 mM DCIP Standard Solution into a 96 well plate, generating 0 (blank), 8, 16, 24, 32, and 40 nmole/well standards. Add SDH Assay Buffer to each well to bring the volume to 100 µL.

Sample Preparation
Tissue samples (10 mg) or cells ($1 \times 10^6$) can be homogenized in 100 µL of ice cold SDH Assay Buffer. Keep on ice for 10 minutes. Centrifuge the samples at 10,000 × *g* for 5 minutes to remove insoluble material. Transfer supernatant to a fresh tube.

Notes: When analyzing SDH activity in mitochondria, it is recommended to isolate the mitochondria from fresh tissue or cells.

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

For biological samples exhibiting significant background, include a sample matrix Blank for each sample by omitting the SDH Substrate Mix. The sample matrix blank readings can then be subtracted from the sample readings.

Add 5–50 µL of the samples into duplicate wells. Bring samples to a final volume of 50 µL using SDH Assay Buffer.

For a positive control (optional), add 10–20 µL of the SDH Positive Control solution to the desired wells. Adjust the final volume to 50 µL with the SDH Assay Buffer.

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

**Table 1.**
Reaction Mixes

| Reagent | Samples and Positive Control | Sample Blank |
|---|---|---|
| SDH Assay Buffer | 46 µL | 48 µL |
| SDH Substrate Mix | 2 µL | – |
| SDH Probe | 2 µL | 2 µL |

2. Add 50 µL of the appropriate Reaction Mix to each of the wells. Do not add Reaction Mix to the DCIP Standard wells. Mix well using a horizontal shaker or by pipetting.

3. Incubate the plate at 25 °C. Take the initial measurement. Measure the absorbance at 600 nm [$(A_{600})_{initial}$] at the initial time ($T_{initial}$).

4. Continue to incubate the plate at 25 °C taking measurements ($A_{600}$) every 5 minutes for 10–30 minutes.

Note: Incubation time depends on the activity of SDH in the samples.

5. Continue taking measurements until the value of the most active sample is greater than the value of the highest standard (40 nmole/well). At this time the most active sample is near or exceeds the end of the linear range of the standard curve.

6. The final absorbance measurement [$(A_{600})_{final}$] for calculating the enzyme activity would be the penultimate reading or the value before the most active sample is near or exceeds the end of the linear range of the standard curve (see step 5). The time of the penultimate reading is $T_{final}$.

Note: It is essential that $(A_{600})_{final}$ falls within the linear range of the standard curve.

The DCIP Standards can be read at the end of the incubation time.

**Results**

Calculations

Correct for the background by subtracting the final measurement [$(A_{600})_{final}$] obtained for the 0 (blank) DCIP Standard from the final measurement [$(A_{600})_{final}$] of the standards and samples. Background values can be significant and must be subtracted from all readings. Use the values obtained from the appropriate DCIP Standards to plot a standard curve.

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

Calculate the change in absorbance measurement from $T_{initial}$ to $T_{final}$ for the samples.

$$\Delta A_{600} = (A_{600})_{initial} - (A_{600})_{final}$$

Subtract the Sample Blank $\Delta A_{600}$ value from the Sample $\Delta A_{600}$ reading to obtain the corrected measurement. Using the corrected measurement, determine the amount of DCIP (nmole/well) generated by the SDH assay between $T_{initial}$ and $T_{final}$ ($S_a$).

SDH activity:

$$\text{SDH Activity} = \frac{S_a}{(\text{Reaction Time}) \times S_v}$$

where:

$S_a$ = Amount of DCIP (nmole) generated in unknown sample well between $T_{initial}$ and $T_{final}$ from standard curve

Reaction Time = $T_{final} - T_{initial}$ (minutes)

$S_v$ = sample volume (μL) added to well

SDH activity is reported as nmole/min/μL = milliunit/μL. One unit of succinate dehydrogenase is the amount of enzyme that generates 1.0 μmole of DCIP per minute at pH 7.2 at 25 °C.

Sample Calculation:

Amount of DCIP ($S_a$) = 15.84 nmole (from standard curve)

($T_{initial}$) = 3 minutes

($T_{final}$) = 32 minutes

Sample volume ($S_v$) = 50 μL

SDH activity in sample well:

$$\text{nmole/min/}\mu\text{L} = \frac{15.84 \text{ nmole/well}}{(32 \text{ min} - 3 \text{ min}) \times 50 \ \mu\text{L/well}} = 0.0109$$

(milliunits/μL)

**References**


1. Kim, H.J., and Winge, D.R., Emerging concepts in the flavinylation of succinate dehydrogenase. Biochim. Biophys. Acta, **1827**, 627–636 (2013).
2. Rutter, J. et al., Succinate dehydrogenase – Assembly, regulation and role in human disease. Mitochondrion, **10**, 393–401 (2010).
3. Bardellad, C. et al., SDH mutations in cancer. Biochim. Biophys. Acta, **1807**, 1432–1443 (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 needed to use 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 Reaction Mixes 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 Mixes |
| | Pipetting errors in preparation of standards | Avoid pipetting small volumes |
| | Pipetting errors in the Reaction Mix | Prepare a Reaction Mixes whenever possible |
| | Air bubbles formed in well | Pipette gently against the wall of the plate well |
| | 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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