Technical Bulletin

Succinate Dehydrogenase Assay Kit

Catalogue number MAK561

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 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 colorimetric assays in 96-well plates.

- SDH Assay Buffer 25 mL
  Catalogue Number MAK561A
- SDH Substrate Mix 0.6 mL
  Catalogue Number MAK561B
- SDH Probe 0.6 mL
  Catalogue Number MAK561C
- DCIP Standard 0.4 mL
  Catalogue Number MAK561D
- SDH Positive Control 1 Vial
  Catalogue Number MAK561E

Reagents and Equipment Required but Not Provided

- 96-well plates, clear, flat bottom. It is recommended to use clear plates for colorimetric assays.
- Plate reader that is capable to read wavelength of 600 nm.
- Pipettors and Pipettes
- Vortex Mixer
- Mitochondria Isolation Kit (optional for mitochondria samples, Catalogue Number MITOISO1 for tissue, MITOISO2 for cells, MITOISO3 for yeast, or equivalent).
- Tergitol (catalogue number T1135) Optional to add 1% Tergitol to SDH buffer for extracting mitochondria from cells or tissue.

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.

Storage/Stability

The product is shipped on wet ice. Store at -20 °C upon receipt.

Preparation Instructions

Briefly centrifuge vials before opening. Use ultrapure water for the preparation of reagents. Avoid repeated freeze/thaw cycles.

SDH Assay Buffer: Allow buffer to come to room temperature.

SDH Substrate Mix: Allow mixture to come to room temperature.

SDH Probe: Allow to thaw (water bath at 30 °C may be used).

DICP Standard: Allow to thaw (water bath at 30 °C may be used).

SDH Positive Control: Reconstitute with 500 µL of ultrapure water. Mix well by pipetting. Aliquot and store at –80 °C. Keep on ice during use.

Procedure

All Samples and Standards should be run in technical triplicates.

Sample Preparation

Liquid samples may be assayed directly.

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.

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

1. Tissue samples (10 mg) or cells (10^6) can be homogenized in 100 µL of ice-cold SDH Assay Buffer containing 1% Tergitol.
2. Keep on ice for 10 minutes.
3. Centrifuge the samples at 10,000 g for 5 minutes to remove insoluble material.
4. Transfer supernatant to fresh tube.

Alternatively, MITOIOS1 and MITOISO2 may be used for mitochondria extraction from tissue or cell samples.

Preparation of DCIP Standards

1. Add 0, 4, 8, 12, 16, and 20 µL of the 2 mM DCIP Standard Solution (MAK561E) into separate wells generating 0 (blank), 8, 16, 24, 32, and 40 nmole/well standards.
2. Add Assay Buffer to each well to bring the final volume per well to 100 µL as in Table 1.

Table 1.
Preparation of DCIP Standards

DCIP STD	Assay Buffer	DCIP Conc.
0 µL	100 µL	0 nmol
4 µL	96 µL	8 nmol
8 µL	92 µL	16 nmol
12 µL	88 µL	24 nmol
16 µL	84 µL	32 nmol
20 µL	80 µL	40 nmol

Positive Control

For a positive control (optional), add 20–50 µL of the SDH Positive Control solution to the desired wells. Adjust the final volume to 90 µL with Assay Buffer.

Assay Reaction

Note: Prepare the reaction mix immediately before use.

For your convenience, a downloadable assay calculator is available on the MAK561 product page and may be used to calculate the amounts needed for the reaction.

1. Prepare the reaction mix by mixing the SDH Substrate and SDH Probe in a 1:1 ratio. 10 µL reaction mix is needed per well.
2. Add the samples to separate wells of a 96-well plate and bring to a volume of 90 µL using Assay Buffer.
3. Add 10 µL reaction mix to only the sample and positive control wells. Do not add the reaction mix to the DCIP standard wells.

Table 2.

Example of a Sample Reaction Set-up

Reagent	Samples and Positive Control	Sample Blank
SDH Assay Buffer	40 µL	40 µL
Reaction Mix	10 µL	-
SDH Substrate Mix	-	10 µL
Sample	50 µL	50 µL

4. Incubate the plate at 25 °C. Measure the absorbance at 600 nm at the initial time ($T_{Initial}$).
5. Take measurements (A600) every 3 minutes for 10–30 minutes.

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

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

Results

Calculations

Correct for the background by subtracting the final measurement [T_{final}] obtained for the 0 (blank) DCIP Standard from the final measurement [$(A600)_{final}$] of the standards and samples. Background values can be significant and must be subtracted from all readings. Use the values obtained from the appropriate blank.

Use the values obtained from the standards to plot a standard curve.

Note: A new standard curve must be set up each time.

Calculate the change in absorbance measurement from $T_{Initial}$ to T_{Final} for the samples.

$\Delta A600 = (A600)_{initial} - (A600)_{final}$

Subtract the Sample Blank DA600 value from the Sample DA600 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} (Sa).

$$\text{SDH activity} = \frac{Sa \times D}{(\text{Reaction time}) \times Sv}$$

Where:

Sa = Amount of DCIP (nmole) generated in unknown sample well between $T_{initial}$ and T_{final} from standard curve (subtract sample blank - only single point needed).

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

Sv = sample volume (µL) added to the well

D = Dilution factor

SDH activity is reported as:

nmole x min^{-1} x μL^{-1} = milliunit x μL^{-1}

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.

Example Calculation:

Sa (A600) initial - (A600) final = 6.34 nmole.

Reaction time $T_{final} - T_{initial}$: 3-18 = 15 minutes

Sv = 50 µL

D = 1

$$\text{SDH activity} = \frac{6.34 \times 1}{15 \times 50} = 0.00845 \frac{\text{nmol}}{\text{min} \times \mu L}$$

SDH activity in well: 0.00845 milliunits/µL

Figure 1.
An exemplary standard curve.

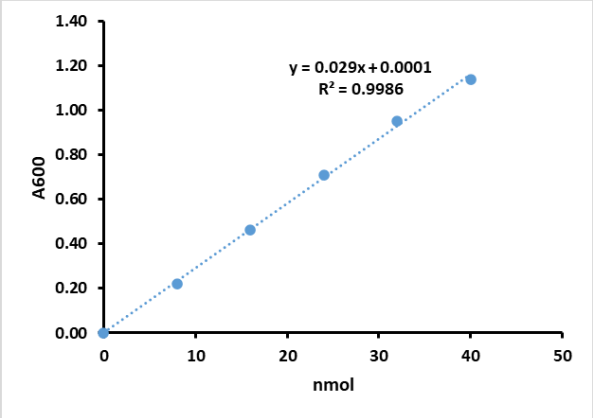
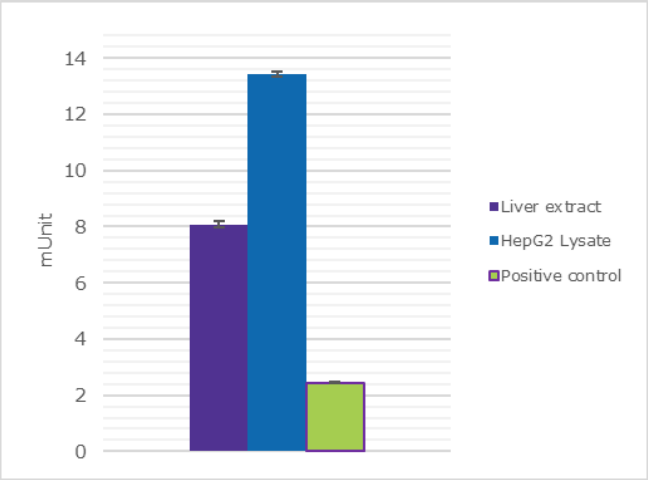


Figure 2.

Purple: Liver (1 g) was homogenized in ice cold 1 mL SDH +1% Tergitol buffer using ULTRA-TURRAX®.
Blue: HepG2 cell lysate ($7x10^6$ cells) was homogenized in ice cold 0.7mL SDH +1% Tergitol.
Green: Positive control.



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 flat bottom, 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 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 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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