

Version 5b, Last updated 2 February 2026

ab228560 Succinate Dehydrogenase Assay Kit (Colorimetric)

For the measurement of Succinate Dehydrogenase activity in isolated mitochondria and in tissues and both adherent and suspension cells

This product is for research use only and is not intended for diagnostic use.

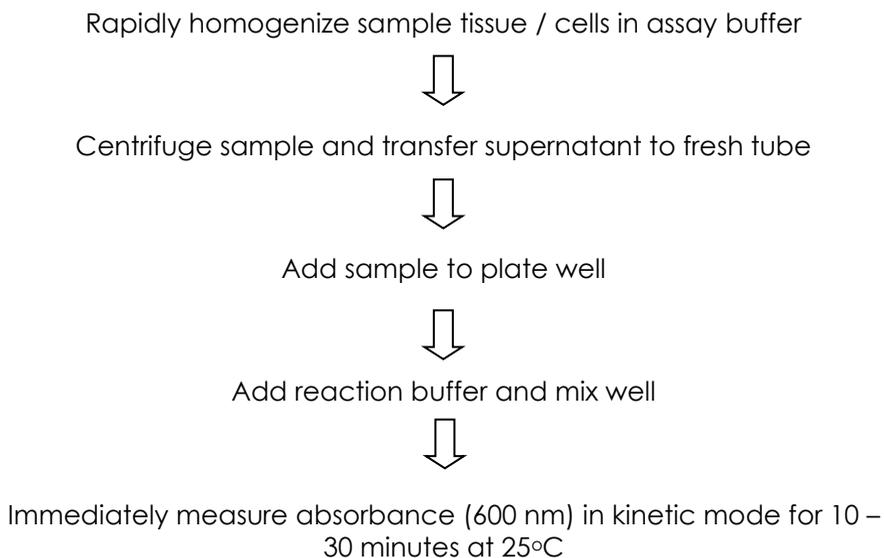
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1. Overview

Succinate Dehydrogenase Activity Assay Kit (Colorimetric) (ab228560) is rapid, simple and high-throughput adaptable. In this assay, Succinate Dehydrogenase converts succinate to fumarate, and transfers the electron to an artificial electron acceptor (Probe), which changes the color from blue to a colorless product. This assay kit can detect less than 0.1 mU Succinate Dehydrogenase activity in a variety of samples.

The kit provides enough reagents for 100 assays using the methods as described.



2. Materials Supplied and Storage

Store kit at -20°C in the dark immediately on receipt and check below for storage for individual components. Kit can be stored for 1 year from receipt, if components have not been reconstituted.

Aliquot components in working volumes before storing at the recommended temperature.

Item	Quantity	Storage temperature (before prep)	Storage temperature (after prep)
SDH Assay Buffer	25 mL	-20°C	-20°C or 4°C.
SDH Substrate Mix	1 vial	-20°C	-20°C (avoid repeated freeze-thaws)
Electron Probe	0.2 mL	-20°C	-20°C
DCIP Standard	0.4 mL	-20°C	-20°C
SDH Positive Control	1 vial	-20°C	-80°C (aliquot after reconstitution and avoid freeze-thaw)

3. Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully perform this assay:

- Microplate reader capable of measuring absorbance at 600 nm in kinetic mode
- 96 well plate with clear flat bottom
- Dounce homogenizer
- (Optional) Protease inhibitors: we recommend Protease Inhibitor Cocktail II (ab201116) [AEBSF, aprotinin, E-64, EDTA, leupeptin] as general use cocktail. Add prior to sample homogenization

4. General guidelines, precautions, and troubleshooting

Please observe safe laboratory practice and consult the safety datasheet.

For general guidelines, precautions, limitations on the use of our assay kits and general assay troubleshooting tips, particularly for first time users, please consult our guide:

www.abcam.com/assaykitguidelines

For typical data produced using the assay, please see the assay kit datasheet on our website.

5. Reagent Preparation

Briefly centrifuge small vials at low speed prior to opening.

5.1 **SDH Assay Buffer:**

Ready to use as supplied. Equilibrate to room temperature before use.

5.2 **SDH Substrate Mix:**

Reconstitute with 220 μ L dH₂O. Aliquot and store at -20°C. Avoid repeated freeze-thaw. Keep on ice whilst in use.

5.3 **Electron Probe:**

Ready to use as supplied. Warm to room temperature before use.

5.4 **DCIP Standard:**

Ready to use as supplied. Warm to room temperature before use

5.5 **SDH Positive Control:**

Reconstitute with 100 μ L SDH Assay Buffer. Aliquot and store at -80°C. Avoid repeated freeze-thaw. Keep on ice during use.

6. Standard Preparation

- Always prepare a fresh set of standards for every use.
 - Discard working standard dilutions after use as they do not store well.
1. The DCIP Standard is ready to use.
 2. Using the DCIP Standard, prepare standard curve dilution as described in the table in a 96-well microplate:

Standard#	DCIP Standard (2 mM) (μL)	SDH Assay Buffer (μL)	Final volume standard in well (μL)	End amount DCIP Standard in well (nmol/well)
1	0	100	100	0
2	4	96	100	8
3	8	92	100	16
4	12	88	100	24
5	16	84	100	32
6	20	80	100	40

7. Sample Preparation

General sample information:

- We recommend performing several dilutions of your sample to ensure the readings are within the standard value range.
- We recommend that you use fresh samples for the most reproducible assay.
- If you cannot perform the assay at the same time, we suggest that you snap freeze your samples in liquid nitrogen upon extraction and store them immediately at -80°C . When you are ready to test your samples, thaw them on ice. Be aware, however, that this might affect the stability of your samples and the readings can be lower than expected. Avoid multiple freeze-thaws. The user will need to verify that samples retain activity upon snap freezing/thawing.
- For certain samples, it may be advantageous to add protease inhibitors: we recommend Protease Inhibitor Cocktail II (ab201116) [AEBSF, aprotinin, E-64, EDTA, leupeptin] as a general use cocktail. Add prior to sample homogenization

7.1 Tissues:

1. Harvest the tissue of interest.
2. Rapidly homogenize tissue (10 mg) in 100 μL ice-cold SDH Assay Buffer.
3. Keep on ice for 10 minutes.
4. Centrifuge at 10,000 x g for 10 minutes and transfer the supernatant to a fresh tube.

7.2 Cells:

1. Harvest the cells of interest.
2. Wash with ice-cold PBS.
3. Rapidly homogenize cells (1×10^6) in 100 μL ice-cold SDH Assay Buffer.
4. Centrifuge at 10,000 x g for 5 minutes and transfer the supernatant to a fresh tube.

7.3 Mitochondria:

1. Use an appropriate method to isolate mitochondria from fresh tissue or cells.
2. Use the isolated mitochondria as the source of Succinate Dehydrogenase.

8. Assay Procedure

- Assay all standards, controls and samples in duplicate.

8.1 Preliminary

1. Prepare SDH Positive Control (Section 5.5).
2. Prepare DCIP Standard solution (Section 7).
3. Prepare sample(s) (Section 8).
4. Equilibrate SDH Assay Buffer (Section 6).

8.2 Reaction wells set up:

- Standard wells = 100 μ L Standard dilutions.
- Sample wells = 5 – 50 μ L sample (adjust volume to 50 μ L/well with SDH Assay Buffer).
- Positive control wells = 10 – 20 μ L SDH Positive Control (adjust final volume to 50 μ L with SDH Assay Buffer).

8.3 SDH Reaction mix:

1. Prepare 50 μ L of SDH Reaction Mix for each reaction (sample and positive controls). Prepare a master mix to ensure consistency.

Component	Reaction Mix (μ L)
SDH Assay Buffer	46
Electron Probe	2
SDH Substrate Mix	2

2. Add 50 μ L of SDH Reaction Mix into each positive control and sample well (to give a final volume of 100 μ L).
3. Mix well.
4. Immediately measure absorbance (600 nm) at 25°C in kinetic mode.
5. Follow absorbance change for 10 – 30 minutes.
6. Select 2 time-points (T_1 and T_2) in the linear range to calculate the Succinate Dehydrogenase activity of the samples.
7. The DCIP Standard curve can be read in end-point mode (i.e. at the end of the assay period).

9. Data Analysis

Samples producing signals greater than that of the highest standard should be further diluted in appropriate buffer and reanalyzed, then multiply the concentration found by the appropriate dilution factor.

1. Subtract the 0 DCIP Standard reading from all readings.
2. Plot the DCIP Standard curve.
3. Calculate the Succinate Dehydrogenase activity of the sample ($\Delta OD = A_1 - A_2$).
4. Use the ΔOD value to determine the nmol (B) of DCIP reduced during the reaction time ($\Delta T = T_2 - T_1$).
5. Average the duplicate reading for each Standard, Positive Control and Sample.
- 6.

$$\text{Succinate Dehydrogenase activity} = \frac{B}{(\Delta T * V)} * D$$

Where:

B = amount of DCIP reduced in the sample well calculated from the standard curve (nmol).

V = sample volume added in the sample well (μL).

D = sample dilution factor if sample is diluted to fit within the standard curve range (prior to reaction well set up).

ΔT = reaction time (minutes).

One unit of succinate Dehydrogenase activity = 1.0 μmol of DCIP reduced / minute at pH 7.2 at 25°C.

10. Typical Data

Typical standard curve and assay data. Data provided for **demonstration purposes only**. A new standard curve must be generated for each assay performed.

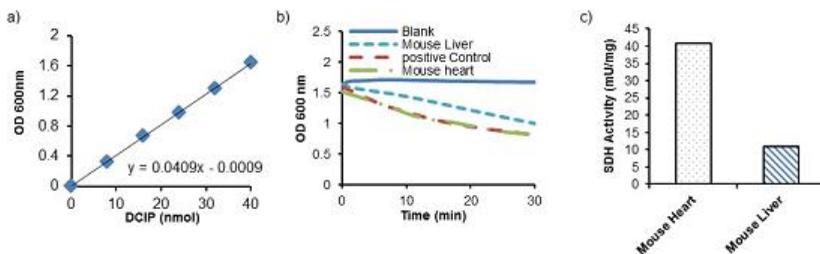


Figure 1. Typical SDH standard curve and assay data. (a) DCIP standard curve, (b) typical assay data and (c) succinate dehydrogenase activity in mitochondria isolated from mouse heart (24 μ g) and liver (70 μ g).

11. Notes

Technical Support

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