Succinate Dehydrogenase (SDH) Activity Assay Kit (Colorimetric)

Cat #: orb1173193 (manual)

Product name: Succinate Dehydrogenase (SDH) Activity Assay Kit (Colorimetric)

Catalog number: orb1173193

Applicable samples: Animal and Plant Tissues, Cells and Bacteria

Storage: Stored at -20°C for 6 months, protected from light

Assay Principle

Succinate Dehydrogenase (SDH) is widely present in animals, plants, microorganisms and cultured cells. SDH is a marker enzyme of mitochondria and a membrane-bound enzyme located on the inner membrane of mitochondria. Also, it is one of the hubs to connect respiratory electron transfer and oxidative phosphorylation. In addition, SDH can provide electrons for the respiratory chain of various prokaryotic cell capacity. Succinate Dehydrogenase (SDH) Activity Assay Kit (Colorimetric) provides a simple method for detecting SDH activity in animal and plant tissues, cells and bacteria. The detection principle is based on that SDH can catalyze succinate to dehydrogenate and produce fumaric acid. The hydrogen can be transmitted by Phenazine dimethyl ester sulphuric acid (PMS), thus reducing 2,6-Dichlorophenolindophenol (2,6-DCPIP) and 2,6-DCPIP has a special absorption peak at 605 nm. The reducing speed of 2,6-DCPIP can be determined by detecting the change of absorbance at 605 nm, then SDH activity in samples can be calculated.

Materials Supplied and Storage Conditions

Kit components	Size	Storage conditions
	96 T	
Extraction Buffer I	60 mL×2	4°C
Extraction Buffer II	1.5 mL	-20°C, protected from light
Reagent I	Powder×1 vial	4°C, protected from light
Reagent II	Powder×1 vial	-20°C, protected from light

Note: Before formal testing, it is recommended to select 2-3 samples with large expected differences for pre-experiment.

Materials Required but Not Supplied

- Microplate reader or visible spectrophotometer capable of measuring absorbance at 605 nm
- 96-well plate or microglass cuvette, precision pipettes, disposable pipette tips
- Refrigerated centrifuge, water bath, ice maker

- Deionized water
- Homogenizer (for tissue samples)

Reagent Preparation

Extraction Buffer Ⅰ: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4°C.

Extraction Buffer Ⅱ: Ready to use as supplied. Stored at -20°C, protected from light.

Reagent Ⅰ: Before use, add 21.6 mL deionized water to fully dissolve. The prepared reagent can be stored at 4°C, protected from light for 1 month.

Note: A small amount of precipitate in Reagent Ⅰ is normal. If it affects the results, please filter it.

Reagent Ⅱ: Before use, add 1.2 mL deionized water to fully dissolve. The remaining reagent can also be stored at -20°C and protected from light for 1 month after aliquoting to avoid repeated freezing and thawing.

Sample Preparation

Note: Fresh samples are recommended, If not assayed immediately, samples can be stored at -80 ℃ for one month. Processed samples must be assayed immediately. All samples and reagents should be on ice to avoid denaturation and deactivation.

1. Animal or plant tissues: Weigh 0.1 g tissues, add 1 mL Extraction Buffer Ⅰ and 10 µL Extraction Buffer Ⅱ and homogenize on ice. Centrifuge at 11,000 g for 10 min at 4℃. Use supernatant for assay, and place it on ice to be tested.
2. Cells or bacteria: Collect 5×10^6 cells or bacteria into the centrifuge tube, wash cells or bacteria with cold PBS, discard the supernatant after centrifugation; add 1 mL Extraction Buffer Ⅰ and 10 µL Extraction Buffer Ⅱ to ultrasonically disrupt the cells or

bacteria 5 min on ice (power 20% or 200 W, ultrasonic 3 s, interval 7 s, repeat 30 times). Centrifuge at 11,000 g for 10 min at 4℃.

Use supernatant for assay, and place it on ice to be tested.

Assay Procedure

1. Preheat the microplate reader or visible spectrophotometer for more than 30 min, and adjust the wavelength to 605 nm, visible spectrophotometer was returned to zero with deionized water.
2. Preheated reagent Ⅰ for 10 min in 37℃ (mammal) or 25℃ (other species) water bath.
3. Add 10 µL of sample, 180 µL of Reagent Ⅰ, and then 10 µL of Reagent Ⅱ in a 96-well plate or microglass cuvette. After mixing quickly, record the absorbance values of 20 s, mark as A1 and incubate for 5 min in 37℃ (mammal) or 25℃ (other species), record the absorbance values of 5 min 20 s at 605 nm with a microplate reader, mark as A2, and calculate ΔA=A1-A2.

Note: In order to guarantee the accuracy of experimental results, need to do a pre-experiment with 2-3 samples.

If ΔA_{Test} is less than 0.001, increase the sample quantity appropriately or the reaction time can be extended for 10-15 min.

If ΔA_{Test} is greater than 0.3, the sample can be appropriately diluted with Extraction Buffer Ⅰ, the calculated result multiplied by the dilution factor, or decrease the sample quantity appropriately.

Data Analysis

Note: We provide you with calculation formulae, including the derivation process and final formula. The two are exactly equal. It is suggested that the concise calculation formula in bold is final formula.

A. 96-well plates calculation formula

1. Calculation of SDH activity in tissue of the sample:

Unit definition: an enzyme activity unit defines as 1 g tissue catalyzes the oxidation of 1 nmol 2,6-DCPIP per min in the reaction system at 37℃(mammal) or 25℃ (other species).

$SDH_{Supernatant}$ (U/g weight)=$[\Delta A_{Supernatant}\times V_{Total}\div(\varepsilon\times d)\times 10^9]\div(W\div V_{Extraction\ Buffer}\times V_{Sample})\div T$**=384.76×ΔA÷W**

2. Calculation of SDH activity in cells and bacteria:

Unit definition: an enzyme activity unit defines as 10,000 cells and bacteria catalyze the oxidation of 1 nmol 2,6-DCPIP per min in the reaction system at 37℃(mammal) or 25℃ (other species).

SDH (U/10^4)=$[\Delta A\times V_{Total}\div(\varepsilon\times d)\times 10^9]\div(V_{Sample}\div V_{Extraction\ Buffer}\times 500)\div T$**=0.77×ΔA**

Where: V_{Total}: total reaction volume, 2×10^{-4} L;

ε: 2,6-DCPIP molar extinction coefficient, 2.1×10^4 L/mol/cm;

d: 0.5 cm;

V_{Sample}: sample volume added, 0.01 mL;

$V_{Extraction\ Buffer}$: sample Extraction Buffer volume, 1.01 mL;

T: reaction time, 5 min;

W: sample weight, g; 500: total number of cells or bacteria, 5 million.

B. Microglass cuvette calculation formula

The optical diameter d:0.5 cm in the above calculation formula can be adjusted to d:1 cm for calculation.

Disclaimer

The reagent is only used in the field of scientific research, not suitable for clinical diagnosis or other purposes. For your safety and health, please wear a lab coat and disposable gloves

Biorbyt Ltd
7 Signet Court, Swann Road, Cambridge, CB5 8LA. United Kingdom
Email: info@biorbyt.com | Phone: +44 (0)1223 859-353 | Fax: +44 (0)1223 280-240

Biorbyt LLC
68 TW Alexander Drive, Durham, NC 27713. United States
Email: info@biorbyt.com | Phone: +1 (415) 906-5211 | Fax: +1 (415) 651-8558