EZMeta™ Succinate Dehydrogenase (SDH) Colorimetric Activity Kit

Cat #: D-AKC1230

Size: 96T

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

Product Information

Applicable samples: Animal and Plant Tissues, Cells and Bacteria

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. EZMeta™ Succinate Dehydrogenase (SDH) Colorimetric Activity Kit 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 transmited 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
	48 T	96 T	
Extraction Buffer	50 mL	100 mL	4°C
Reagent Ⅰ	10 mL	20 mL	4°C
Reagent Ⅱ	0.75 mL	1.5 mL	-20°C, protected from light
Reagent Ⅲ	1	1	4°C
Reagent Ⅳ	1	1	-20°C, protected from light

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

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

Reagent Ⅱ: Ready to use as supplied. Equilibrate to room temperature before use. Store at -20°C, protected from light.

ReagentⅢ: Before use, add 9 mL deionized water to fully dissolve for 48 T; add 18 mL deionized water to fully dissolve for 96 T. The remaining reagents should be stored at 4°C.

ReagentⅣ: Before use, add 0.5 mL deionized water to fully dissolve for 48 T; add 1 mL deionized water to fully dissolve for 96 T. The remaining reagents should be stored at -20°C and protected from light 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°C for one month. Processed samples must be assayed immediately. All samples and reagents should be on ice to avoid denaturation and deactivation.

Extraction of cytoplasmic protein and mitochondrial protein from cells, bacteria and tissue:

1. Weigh 0.1 g tissue or collect 5×10^6 cells and bacteria, add 1 mL Extraction Buffer and 10 μL Reagent Ⅱ, homogenize on ice. Centrifuge at 600 g for 5 min at 4°C. Collect the supernatant to a new centrifuge tube and discard the pellet.

2. Centrifuge the supernatant again at 11,000 g for 10 min at 4°C, and obtain the supernatant and precipitate respectively.

3. (Optional) The supernatant collected in step 2 is cytoplasmic extract, which can be used to determine SDH leaking from mitochondria.

4. Add 200 μL Reagent Ⅰ and 2 μL Reagent Ⅱ to the precipitate collected in step 2, resuspend the precipitate sufficiently, and use it to detect the activity of SDH in the next step.

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 reagentⅢ for 10 min in 37°C (mammal) or 25°C (other species) water bath.

3. Add 10 μL of sample, 180 μL of ReagentⅢ, and then 10 μL of ReagentⅣ in a 96-well plate or microglass cuvette. After mixing quickly, record the absorbance values of 20 s and 1 min 20 s at 605 nm with a microplate reader, mark as A_1 and A_2, and calculate $\Delta A=A_1-A_2$.

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. If ΔA_{Test} is greater than 0.3, the sample can be appropriately

diluted with Extraction Buffer, 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°C(mammal) or 25°C(other species).

$SDH_{Supernatant}$ (U/g weight)=$[\Delta A_{Supernatant}\times V_{Total}\div(\varepsilon\times d)\times 10^9]\div(W\div V_{Extraction}\times V_{Sample})\div T$=**$1{,}923.8\times\Delta A_{Supernatant}\div W$**

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

Total SDH (U/g weight)=$SDH_{Supernatant}+SDH_{Precipitate}$=**$1{,}923.8\times\Delta A_{Supernatant}\div W+384.76\times\Delta A_{Precipitate}\div W$**

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°C(mammal) or 25°C(other species).

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

Where: $\Delta A_{Supernatant}$: OD value of supernatant; $\Delta A_{Precipitate}$: OD value of precipitate; 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; $V_{Extraction}$: sample extract volume, 1.01 mL; $V_{Total\ Sample}$: the volume of adding Reagent Ⅰ and Ⅱ, 0.202 mL; T: reaction time, 1 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.

Typical Data

Take 0.1 g of mouse brain tissue, then follow the determination steps, and measure with 96-well plate:

$\Delta A_{Supernatant}=A_1-A_2=0.281-0.256=0.025$; $\Delta A_{Precipitate}=A_1-A_2=0.474-0.444=0.03$;

Calculate the SDH activity according to the weight of the sample:

SDH (U/g weight)=$SDH_{Supernatant}+SDH_{Precipitate}=1923.8\times\Delta A_{Supernatant}\div W+384.76\times\Delta A_{Precipitate}\div W=1923.8\times0.025\div0.1+384.76\times0.03\div0.1=596.38$ U/g weight.

Disclaimer

The reagent is only used in the field of scientific research, not suitable for clinical diagnosis or other purposes.