

EZMeta™ Mitochondrial complex I Colorimetric Activity Kit

Cat #: D-AKC1850

Size: 48T / 96T

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

Product Information

Applicable samples: Animal and Plant Tissues, Cells

Assay Principle

Mitochondrial respiratory chain complex I (EC 1.6.5. 3), also known as NADH-CoQ reductase or NADH dehydrogenase, is the largest protein complex in the mitochondrial intima. Complex I is widely found in the mitochondria of animals, plants, microorganisms and cultured cells. This enzyme catalyzes the transfer of a pair of electrons from NADH to CoQ, and at the same time it can reduce O_2 to produce O_2^- , which is the main part of the respiration electron transport chain to produce O_2^- . The activity of Complex I can not only reflect the status of responsibility electron transfer chain (ETC), but also reflect the production of reactive oxygen species (ROS). EZMeta™ Mitochondrial complex I Colorimetric Activity Kit provides a convenient tool for detection of Mitochondrial complex I Activity. The principle is that Complex I can catalyze the dehydrogenation of NADH to NAD^+ . The oxidation rate of NADH can be determined at 340 nm to calculate the activity of Complex I. It can be used to determine animal, plant tissue and cell samples.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	48 T	96 T	
Reagent I	50 mL	100 mL	4°C
Reagent II	10 mL	20 mL	4°C
Reagent III	1 mL	2 mL	4°C, protected from light
Reagent IV	12.5 mL	25 mL	-20°C, protected from light
Reagent V	0.5 mL	1 mL	-20°C, protected from light
Reagent VI	1	1	-20°C, protected from light

Materials Required but Not Supplied

- Microplate reader or ultraviolet spectrophotometer capable of measuring absorbance at 340 nm
- Incubator, ice maker, refrigerated centrifuge
- 96-well UV plate or microquartz cuvette, precision pipettes, disposable pipette tips
- Deionized water
- Homogenizer (for tissue samples)

Reagent Preparation

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 4°C.

Reagent III: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4°C, protected from light.

Working Reagent VI: Before use, for 48 T add 1 mL deionized water to dissolve it, for 96 T add 2mL deionized water to dissolve it. Please aliquot the unused reagents and store at -20°C, protected from light. Avoid freezing and thawing.

Working Solution: Before use, ReagentIV and Reagent V were mixed at 99:1. Then incubated the mixture at 37°C for 5 min if the detected samples are from mammalian, or incubated at 25 °C for 5 min if the samples are from another species.

Sample Preparation

Note: Fresh samples are recommended to ensure enzyme activity.

Extraction of mitochondrial respiratory chain complex I :

1. Accurately weigh 0.1 g tissue or collect 5×10^6 cells, add 1 mL Reagent I and 10 μ L Reagent III, homogenize on ice.
2. Centrifuge the homogenate with 600 g for 5 min at 4°C, collect the supernatant to a new centrifuge tube and discard the pellet.
3. Centrifuge the supernatant again with 11,000 g for 10 min at 4°C. The pellet is the extracted mitochondria, which could be used to do step 5.
4. (Optional) The supernatant is cytoplasmic extract, which can be used as sample to determine mitochondrial respiratory chain complex I leaking from mitochondria to judge the effect of mitochondrial extraction.
5. Add 200 μ L Reagent II and 2 μ L Reagent III to the pellet, resuspend the pellet sufficiently, and use it to detect the activity of mitochondrial respiratory chain complex I in the next step.

Assay Procedure

1. Preheated the microplate reader or ultraviolet spectrophotometer for more than 30 min, and adjust the wavelength to 340 nm. Ultraviolet Spectrophotometer was returned to zero with deionized water.
2. Add 10 μ L of sample, 200 μ L of Working Solution and 15 μ L of Working Reagent VI to the 96-well UV plate or microquartz cuvette, then tap the plate and mix well. Immediately read the initial 340 nm absorbance value A_1 at 0 min, and then read the absorbance value A_2 after 2 min, 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 1-2 samples. If the absorbance values is too high (above 1.5) or ΔA is greater than 0.4, the samples should be dilute with Reagent II and then measured again. Pay attention to multiply by the dilution factor when calculating the result. If ΔA is too small, the sensitivity can be improved by increasing the sample volume added. If ΔA is negative, it means that complex I is not contained in the sample or has been degraded.

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 UV plates calculation formula

1.Calculated by fresh weight of samples

Unit definition: one enzyme activity unit defines as the consumption of 1 nmol NADH in 1 g tissue reaction system per min.

Calculate the activity of complex I in the supernatant:

The activity of Complex I (U/g fresh weight)=[$\Delta A_1 \times V_{\text{Total}} \div (\epsilon \times d) \times 10^9$] $\div (W \div V_{\text{Extraction}} \times V_{\text{Sample}}) \div T = \mathbf{3,654 \times \Delta A_1 \div W}$

Calculate the activity of complex I of the mitochondrial pellet

The activity of Complex I (U/g fresh weight) = [$\Delta A_2 \times V_{\text{Total}} \div (\epsilon \times d) \times 10^9$] $\div (W \div V_{\text{Resuspended}} \times V_{\text{Sample}}) \div T = \mathbf{731 \times \Delta A_2 \div W}$

Calculate the total activity of complex I in sample:

The total activity of complex I in sample is the sum of the activity of complex I in the supernatant and in the pellet.

Calculated by fresh weight of samples:

Total activity (U/g fresh weight)= $\mathbf{3,654 \times \Delta A_1 \div W + 731 \times \Delta A_2 \div W}$

2.Calculated by cell density

Unit definition: Every 10,000 cells consume 1 nmol NADH per minute is defined as one unit of enzyme activity.

The activity of Complex I (U/ 10^4 cell)=[$\Delta A \times V_{\text{Total}} \div (\epsilon \times d) \times 10^9$] $\div (V_{\text{Sample}} \div V_{\text{Resuspended}} \times 500) \div T = \mathbf{1.46 \times \Delta A}$

Where: V_{Total} : total reaction volume, 2.25×10^{-4} L; ϵ : NADH molar extinction coefficient, 6.22×10^3 L/mol/cm; d: 96-well UV plate diameter, 0.5 cm; 10^9 : Unit conversion factor, 1 mol= 10^9 nmol; V_{Sample} : sample volume added, 0.01 mL; T: reaction time, 2 min; ΔA_1 : determination value of the supernatant; W: sample weight, g; $V_{\text{Extraction}}$: sample extract volume, 1.01 mL; ΔA_2 : determination value of Pellet; $V_{\text{Resuspended}}$: Volume of the resuspend pellet 0.202 mL; 500: Total number of or cells, 5×10^6 .

B. Microquartz 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

Examples:

1. Test 0.1g cabbage, prepared the sample following the above protocol and measured with the 96-well UV microplate:

$$\Delta A_1 = A_1 - A_2 = 0.7819 - 0.7804 = 0.0015. \Delta A_2 = A_1 - A_2 = 0.783 - 0.7704 = 0.0126.$$

2. Calculated by fresh weight of samples

Calculated by fresh weight of samples in supernatant:

$$\text{Complex I activity (U/g fresh weight)} = 3,654 \times \Delta A_1 \div W = 3,654 \times 0.0015 \div 0.1 = 54.81 \text{ U/g.}$$

Calculated by fresh weight of samples in the pellet,

$$\text{Complex I activity (U/g fresh weight)} = 731 \times \Delta A_2 \div W = 731 \times 0.0126 \div 0.1 = 92.106 \text{ U/g.}$$

$$\text{The total Complex I activity (U/g fresh weight)} = 3,654 \times \Delta A_1 \div W + 731 \times \Delta A_2 \div W = 54.81 + 92.106 = 146.916 \text{ U/g.}$$

Disclaimer

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