



## **TECHNICAL MANUAL**

### **Mitochondrial Complex II Activity Assay Kit**

- **SKU CODE:** MAES0223
- **SIZE:** 48 Tests/96 Tests
- **DETECTION PRINCIPLE:** Assay Kit
- **RUO:** Research-Use-Only

## 1. Assay summary

- Reagent preparation
- Sample preparation
- Add reaction working solution and incubate
- Add samples and measure initial OD values
- Incubate and measure final OD values
- Calculate mitochondrial complex II activity

## 2. Intended use

This kit can measure mitochondrial complex II activity in animal tissue samples.

## 3. Detection principle

Mitochondrial complex II, also known as succinate-coenzyme Q reductase, is widely found in the mitochondria of animals, plants, microorganisms, and cultured cells. It catalyzes the oxidation of succinic acid in the TCA cycle to fumaric acid, which converts ubiquinone to a reduced form through the basic unit structures of the complex such as iron-sulfur proteins. Coenzyme Q, the catalytic product of mitochondrial complex II, can further reduce 2,6-dichloroindoxol, which has a characteristic absorption peak at 600 nm. Therefore, the activity of mitochondrial complex II can be quantified by measuring the change in OD value at 600 nm.

## 4. Kit components & storage

| Item      | Component             | Size 1(48 T)    | Size 2(96 T)     | Storage                              |
|-----------|-----------------------|-----------------|------------------|--------------------------------------|
| Reagent 1 | Extraction Solution A | 50 mL × 1 vial  | 50 mL × 2 vials  | -20°C, 12 months                     |
| Reagent 2 | Extraction Solution B | 15 mL × 1 vial  | 30 mL × 1 vial   | -20°C, 12 months                     |
| Reagent 3 | Inhibitor             | 0.8 mL × 1 vial | 0.8 mL × 2 vials | -20°C, 12 months, protect from light |
| Reagent 4 | Buffer Solution       | 10 mL × 1 vial  | 20 mL × 1 vial   | -20°C, 12 months                     |
| Reagent 5 | Substrate A           | 0.8 mL × 1 vial | 1.6 mL × 1 vial  | -20°C, 12 months, protect from light |

| Item      | Component    | Size 1(48 T)    | Size 2(96 T)     | Storage                              |
|-----------|--------------|-----------------|------------------|--------------------------------------|
| Reagent 6 | Substrate B  | 1.5 mL × 1 vial | 1.5 mL × 2 vials | -20°C, 12 months, protect from light |
| Reagent 7 | Substrate C  | 0.6 mL × 1 vial | 1.2 mL × 1 vial  | -20°C, 12 months, protect from light |
|           | Microplate   | 48 wells        | 96 wells         | No requirement                       |
|           | Plate Sealer | 2 pieces        | 2 pieces         |                                      |

## 5. Materials prepared by users

### Instruments:

Microplate reader (590-610 nm, optimum wavelength: 600 nm), Centrifuge

### Reagents:

Normal saline (0.9% NaCl), PBS (0.01 M, pH 7.4)

## 6. Reagent preparation

1. Equilibrate all reagents to room temperature before use.
2. Preparation of reaction working solution: For each well, prepare 190  $\mu$ L of reaction working solution (mix well 150  $\mu$ L of buffer solution, 10  $\mu$ L of substrate A, 20  $\mu$ L of substrate B, and 10  $\mu$ L of substrate C). The reaction working solution should be prepared fresh. Store for 1 day protected from light.

## 7. Sample preparation

Sample preparation - Tissue sample:

1. Harvest the amount of tissue needed for each assay (initial recommendation 100 mg).
2. Wash tissue in cold PBS (0.01 M, pH 7.4).
3. Homogenize 100 mg tissue in 900  $\mu$ L extraction solution A with a dounce homogenizer at 4°C.
4. Centrifuge at 600×g for 5 min, discard the precipitate and take the supernatant.
5. Then centrifuge at 15,000×g for 10 min at 4°C, discard the supernatant and take the precipitate.

6. Mix the precipitate with 200  $\mu\text{L}$  of extraction solution B and 10  $\mu\text{L}$  of inhibitor, sonicate for 1 min at 4°C, then centrifuge at 15,000 $\times g$  at 4°C for 10 min. Take the supernatant for detection.
7. Meanwhile, determine the protein concentration of the supernatant (MAES0177).

Dilution of sample: The recommended dilution factor for different samples is as follows (for reference only):

10% Rat liver tissue homogenate: 1

10% Rat heart tissue homogenate: 1

10% Rat kidney tissue homogenate: 1

10% Mouse liver tissue homogenate: 1

10% Mouse kidney tissue homogenate: 1

10% Mouse heart tissue homogenate: 1

Note: The diluent is extraction solution B. For the dilution of other sample types, please perform a pretest to confirm the dilution factor.

## 8. The key points of the assay

1. When adding the reaction working solution, adhere to the wall and add slowly to avoid bubbles.
2. Use fresh samples for detection. Processed samples should be tested the same day.
3. It is recommended that the number of samples for an experiment be controlled within 10 samples.

## 9. Operating steps

1. Blank well: Add 190  $\mu\text{L}$  of reaction working solution to each well. Sample well: Add 190  $\mu\text{L}$  of reaction working solution to each well.
2. Incubate at 37°C for 3 min.
3. Blank well: Add 20  $\mu\text{L}$  of extraction solution B to blank well. Sample well: Add 20  $\mu\text{L}$  of sample to sample well.
4. Mix thoroughly with microplate reader for 3 s and measure the OD value of each well at 600 nm with microplate reader, recorded as A1.
5. Incubate at 37°C for 3 min, measure the OD value of each well at 600 nm with microplate reader, recorded as A2,  $\Delta A = A1 - A2$ .

## 10. Calculation

For tissue samples:

**Definition:** The amount of mitochondrial complex II in 1 g tissue protein per 1 minute that hydrolyzes the substrate to produce 1  $\mu\text{mol}$  product at room temperature is defined as 1 unit.

$$\text{Mitochondrial complex II activity (U/gprot)} = (\Delta A_{\text{sample}} - \Delta A_{\text{blank}}) \times V_{\text{total}} \times f / (V_{\text{sample}} \times 21.8 \times T \times C_{\text{pr}}) \times 1000$$

[Note]

$\Delta A_{\text{sample}}$ : The change OD value of sample ( $A_1 - A_2$ ).

$\Delta A_{\text{blank}}$ : The change OD value of blank ( $A_1 - A_2$ ).

f: Dilution factor of sample before test.

$V_{\text{total}}$ : The volume of the reaction system, 0.21 mL.

$V_{\text{sample}}$ : The volume of the sample, 0.02 mL.

21.8: Molar absorption coefficient.

$C_{\text{pr}}$ : The concentration of protein in sample, gprot/L.

T: The time of reaction, 3 min.

1000: 1 mmol/L = 1000  $\mu\text{mol/L}$ .

## 11. Appendix I Performance Characteristics

Parameters:

### Intra-assay Precision

Three rat heart tissue samples were assayed in replicates of 20 to determine precision within an assay. (CV = Coefficient of Variation)

| Parameters | Sample 1 | Sample 2 | Sample 3 |
|------------|----------|----------|----------|
| Mean (U/L) | 2.35     | 12.60    | 15.50    |
| %CV        | 4.3      | 3.8      | 3.9      |

### Inter-assay Precision

Three rat heart tissue samples were assayed 20 times in duplicate by three operators to determine precision between assays.

| Parameters | Sample 1 | Sample 2 | Sample 3 |
|------------|----------|----------|----------|
| Mean (U/L) | 2.35     | 12.60    | 15.50    |
| %CV        | 4.9      | 5.2      | 4.9      |

## Recovery

Three samples of high concentration, middle concentration, and low concentration were tested with each concentration tested 6 times in parallel to obtain an average recovery rate of 97%.

| Sample 1 | Sample 2 | Sample 3 |
|----------|----------|----------|
| 4.5      | 9.8      | 14.5     |
| 4.5      | 9.6      | 13.3     |
| 101      | 98       | 92       |

## Sensitivity

The analytical sensitivity of the assay is 0.54 U/L. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 20 times, and calculating the corresponding concentration.

## 12. Appendix II Example Analysis

### Example analysis:

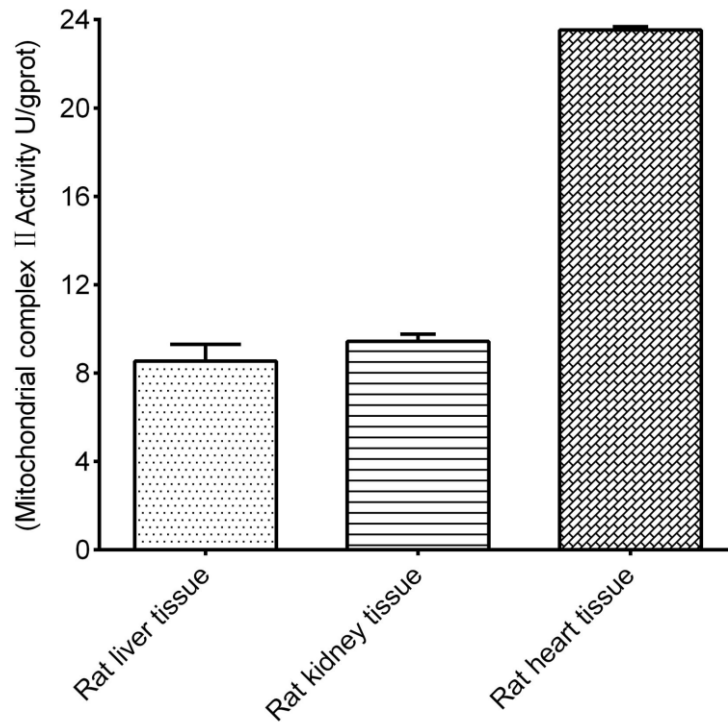
For rat heart tissue, take 20  $\mu$ L of 10% rat heart tissue homogenate, and perform the assay according to the operation steps. The results are as follows:

The OD value of the blank  $A_1$  is 0.979, the OD value of the blank  $A_2$  is 0.972, the OD value of the sample  $A_1$  is 0.671, the OD value of the sample  $A_2$  is 0.310, the concentration of protein in sample is 2.07 gprot/L, and the calculation result is:

Mitochondrial complex II activity

$$(U/gprot) = (0.671 - 0.310) - (0.979 - 0.972) \times 0.21 / (0.02 \times 21.8 \times 3 \times 2.07) \times 1000 = 27.61 \text{ U/gprot}$$

Detect 10% rat liver tissue homogenate (the concentration of protein is 10.29 gprot/L), 10% rat kidney tissue homogenate (the concentration of protein is 10.09 gprot/L), 10% rat heart tissue homogenate (the concentration of protein is 2.07 gprot/L) according to the protocol, the result is as follows:



### 13. Statement

1. This assay kit is for Research Use Only. Assay Genie assumes no responsibility for any problems or legal liabilities arising from the use of this kit for clinical diagnosis or any other purpose.
2. Please read the instructions carefully and calibrate the instruments before performing the experiments. Follow the instructions strictly throughout the procedure.
3. Appropriate protective measures must be taken, including wearing a lab coat and latex gloves.
4. If the concentration of the substance falls outside the detection range, perform an additional dilution or concentration step on the sample.
5. It is recommended to perform a pre-test if your sample type is not listed in the instruction manual.
6. Experimental results are closely related to reagent quality, operator technique, environmental conditions, and other factors. Assay Genie guarantees the quality of the kits only and is NOT responsible for sample consumption resulting from use of the assay kits. It is advisable to estimate the expected sample usage and reserve sufficient samples before starting the experiment.

**Assay Genie 100% money-back guarantee!**

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**Manufacturers Statement: This final kit system is assembled and quality-released by Assay Genie Limited.**