



## **TECHNICAL MANUAL**

# **Mitochondrial Complex III (Coenzyme Q-Cytochrome c Reductase) Activity Colorimetric Assay Kit**

- **SKU CODE:** MAES0241
- **SIZE:** 48 Tests/96 Tests
- **DETECTION PRINCIPLE:** Assay Kit
- **RUO:** Research-Use-Only

## 1. Assay summary

- Sample preparation
- Sample pretreatment
- Add samples and reagents to wells
- Add substrate A and mix
- Measure OD values at 10s and 70s

## 2. Intended use

This kit can measure mitochondrial complex III (Coenzyme Q-Cytochrome C Reductase) activity in animal tissue samples.

## 3. Detection principle

As an organelle, mitochondria serves as the "power factory" in cells and is the main site of cellular aerobic respiration. Its function is to convert energy through oxidative phosphorylation to provide energy for cellular activities. The oxidation process is carried out by four respiratory chain membrane protein complexes (complexes I, II, III and IV) on the inner mitochondrial membrane.

Mitochondrial complex III, also known as cytochrome c reductase complex, functions primarily to oxidize the reduced coenzyme Q10 formed by mitochondrial complexes I and II to oxidized coenzyme Q10. In this process, the OD value increases at 550 nm. Therefore, the activity of mitochondrial complex III can be quantified by measuring the change in OD value at 550 nm.

## 4. Kit components & storage

Item	Component	Size 1(48 T)	Size 2(96 T)	Storage
Reagent 1	Extraction Solution A	55 mL x 1 vial	55 mL x 2 vials	-20 °C, 12 months
Reagent 2	Extraction Solution B	13 mL x 1 vial	26 mL x 1 vial	-20 °C, 12 months

Item	Component	Size 1(48 T)	Size 2(96 T)	Storage
Reagent 3	Extraction Solution C	1.2 mL x 1 vial	1.2 mL x 2 vials	-20 °C, 12 months, shading light
Reagent 4	Substrate A	3 mL x 1 vial	6 mL x 1 vial	-20 °C, 12 months, shading light
Reagent 5	Diluent	7 mL x 1 vial	14 mL x 1 vial	-20 °C, 12 months
Reagent 6	Substrate B	0.8 mL x 1 vial	1.6 mL x 1 vial	-20 °C, 12 months, shading light
Reagent 7	Stabilizer	Powder x 3 vials	Powder x 6 vials	-20 °C, 12 months, shading light
Reagent 8	Buffer Solution	13 mL x 1 vial	26 mL x 1 vial	-20 °C, 12 months
Reagent 9	Inhibitor Controlled Solution	1.5 mL x 1 vial	3 mL x 1 vial	-20 °C, 12 months, shading light
Reagent 10	Inhibitor	1.5 mL x 1 vial	3 mL x 1 vial	-20 °C, 12 months, shading light
	Microplate	48 wells	96 wells	No requirement
	Plate Sealer	2 pieces	2 pieces	

## 5. Materials prepared by users

### Instruments:

Centrifuge, Microplate reader (540-560 nm, optimum wavelength: 550 nm)

## 6. Reagent preparation

1. Equilibrate all reagents to room temperature before use.
2. Preparation of substrate B before use: Place substrate B at 37 °C for 10 min before use and mix the solution until it is clarified for use. Aliquot and store at -20 °C; avoid repeated freeze/thaw cycles.
3. Preparation of stabilizer working solution: Dissolve one vial of stabilizer with 2 mL of diluent, mix well. Store on ice protected from light and use within 6 hours.
4. Preparation of reaction working solution: Before testing, prepare sufficient reaction working solution according to the number of test wells. For example,

prepare 15  $\mu\text{L}$  of reaction working solution by mixing 5  $\mu\text{L}$  of stabilizer working solution with 10  $\mu\text{L}$  of substrate B. Allow the prepared solution to stand at room temperature protected from light for 3 min, then use immediately. The reaction working solution is a white suspension and should be used within 30 min.

## 7. Sample preparation

### 1. Tissue sample preparation:

- 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  $\mu\text{L}$  extraction solution A with a dounce homogenizer at 4 °C.
- 4) Centrifuge at 600 xg for 5 min, discard the precipitate and take the supernatant.
- 5) Then centrifuge at 15,000 xg 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 extraction solution C, sonicate for 1 min, centrifuge at 11,000 xg at 4 °C for 10 min. Then take the supernatant for detection.
- 7) Meanwhile, determine the protein concentration of supernatant (MAES0177).

- 2. Sample dilution:** The recommended dilution factor for different samples is as follows (for reference only): 10% Rat liver tissue homogenate: 1-2 10% Rat kidney tissue homogenate: 1-2 10% Rat brain tissue homogenate: 1 10% Rat lung tissue homogenate: 1 10% Rat spleen tissue homogenate: 1 10% Mouse liver tissue homogenate: 1-2 10% Mouse kidney tissue homogenate: 1-2 10% Mouse brain tissue homogenate: 1 10% Mouse lung tissue homogenate: 1 10% Mouse spleen tissue homogenate: 1 Note: The diluent is extraction solution B. For dilution of other sample types, please perform a pretest to confirm the dilution factor.

## 8. The key points of the assay

1. The mitochondrial samples should be detected within 4 hours if possible. If samples are stored for a long time, the specific enzyme activity will be reduced, and the sample determination results will be low.
2. Detection should begin approximately 10 seconds after adding substrate A, and it is recommended to measure no more than 4 samples at the same time.

3. The change in OD value of the blank well should be within  $\pm 0.005$ , indicating that the reagents are available. If it exceeds this range, check whether substrate B is clear and extend the incubation time.

## 9. Operating steps

- 1. Sample pretreatment:** Total enzyme activity sample: Mix the sample and inhibitor controlled solution at a volume ratio of 20  $\mu\text{L}$ :20  $\mu\text{L}$  completely. Allow the prepared solution to stand at room temperature protected from light for 5 min before use. Non-specific enzyme activity sample: Mix the sample and inhibitor at a volume ratio of 20  $\mu\text{L}$ :20  $\mu\text{L}$  completely. Allow the prepared solution to stand at room temperature protected from light for 5 min before use.
- 2. Measurement of samples:** 1) Add 10  $\mu\text{L}$  of reaction working solution to all wells (blank, total enzyme activity sample, and non-specific enzyme activity sample wells). 2) Add 90  $\mu\text{L}$  of buffer solution to each well. 3) Add samples: Blank well - 20  $\mu\text{L}$  of extraction solution B; Total enzyme activity sample well - 20  $\mu\text{L}$  of total enzyme activity sample; Non-specific enzyme activity sample well - 20  $\mu\text{L}$  of non-specific enzyme activity sample. 4) Add 40  $\mu\text{L}$  of substrate A to each well. Mix fully with microplate reader for 5 s. (Due to the rapid enzymatic reaction, it is recommended to use a multi-channel pipette for adding substrate A, and measure no more than 4 samples at the same time when using an ordinary pipette.) 5) Measure the OD value of each well at 10 s and 70 s respectively at 550 nm with microplate reader, recorded as A1, A2,  $\Delta A = A2 - A1$ .

## 10. Calculation

For tissue sample:

**Definition:** The amount of mitochondrial complex III in 1 g tissue mitochondria protein per 1 minute that reduces 1  $\mu$ mol cytochrome c at room temperature is defined as 1 unit.

mitochondrial complex III activity

$$(U/gprot) = (\Delta A_{Total} - \Delta A_{non-specific}) \times V_1 \times f / [V_2 \times (\epsilon \times d) \times T] \div C_{pr}$$

[Note]

$\Delta A_{Total}$ : The change in OD value of total enzyme activity sample well ( $A_2 - A_1$ ).

$\Delta A_{Non-specific}$ : The change in OD value of non-specific enzyme activity sample well ( $A_2 - A_1$ ).

$V_1$ : The volume of the reaction system, 0.16 mL.

$V_2$ : The volume of the sample, 0.02 mL.

$\epsilon$ : The molar extinction coefficient of cytochrome c at 550 nm, 0.0191 L/ $\mu$ mol/cm

d: Optical path, 0.5 cm

T: The time of reaction, 1 min.

f: Dilution factor of sample before test.

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

## 11. Appendix I Performance Characteristics

Parameter:

### Intra-assay Precision

Three rat brain 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)	10.50	56.00	88.50
%CV	5.2	4.9	4.9

## Inter-assay Precision

Three rat brain 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)	10.50	56.00	88.50
%CV	9.8	10.2	10.0

## Recovery

Three samples of high, middle, and low concentration were tested with 6 parallel replicates each to obtain an average recovery rate of 100%.

	Sample 1	Sample 2	Sample 3
Expected Conc. (U/L)	22.5	76.3	92.5
Observed Conc. (U/L)	22.7	75.5	92.5
Recovery rate (%)	101	99	100

## Sensitivity

The analytical sensitivity of the assay is 4.45 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

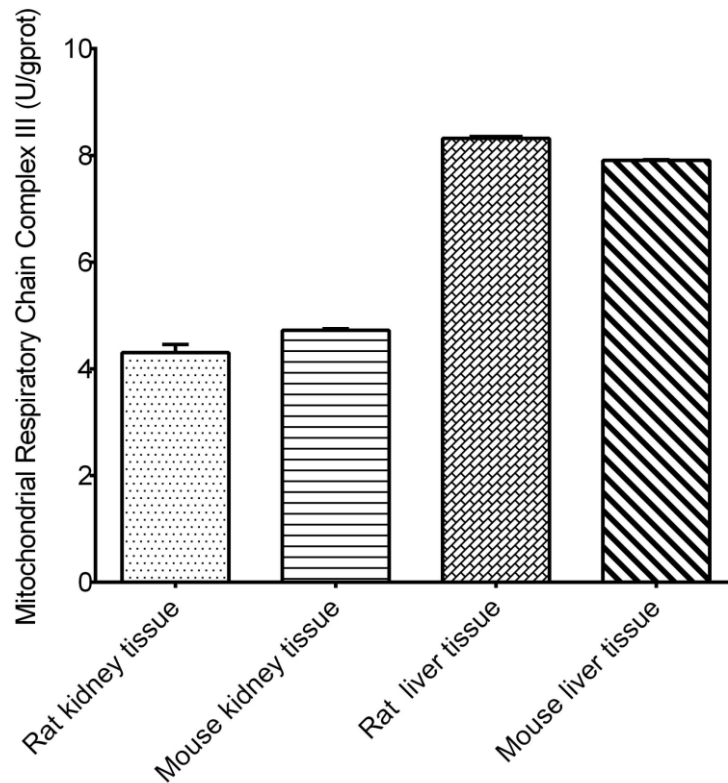
### Example analysis:

For 10% rat kidney tissue mitochondria supernatant, diluted 2-fold, the assay was performed according to the operating steps. The results are as follows:

The  $A_1$  of the blank well is 0.198, the  $A_2$  of the blank well is 0.199,  $\Delta A = 0.001$ , indicating that the reagents are available. The  $A_1$  of total enzyme activity sample well is 0.239, the  $A_2$  of total enzyme activity sample well is 0.306,  $\Delta A_{\text{Total}} = 0.067$ . The  $A_1$  of non-specific enzyme activity sample well is 0.235, the  $A_2$  of non-specific enzyme activity sample well is 0.289,  $\Delta A_{\text{Non-specific}} = 0.054$ . The concentration of mitochondria protein in sample is 5.24 gprot/L, and the calculation result is:

mitochondrial complex III activity (U/gprot) =  $(0.067 - 0.054) \times 0.16 \times 2 / [0.02 \times 0.01917 \times 0.5 \times 1] \div 5.24 = 4.16$  U/gprot

Detected samples: 10% rat kidney tissue homogenate (mitochondria protein concentration 5.24 gprot/L, diluted 2-fold), 10% mouse kidney tissue homogenate (mitochondria protein concentration 6.76 gprot/L), 10% rat liver tissue homogenate (mitochondria protein concentration 4.05 gprot/L, diluted 4-fold) and 10% mouse liver tissue homogenate (protein concentration 5.12 gprot/L) according to the protocol. The results are 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.

**Note:**

**Note:**

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

If you are not satisfied with the quality of our products and our technical team cannot resolve your problem, we will give you 100% of your money back.



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