TECHNICAL MANUAL

Mitochondrial Complex IV (Cytochrome C Oxidase) Activity Assay Kit

- **SKU CODE:** MAES0224
- **SIZE:** 48T / 96T
- **DETECTION PRINCIPLE:** Colorimetric
- **RUO:** Research-Use-Only

Revised: 01/2026

Mitochondrial Complex IV (Cytochrome C Oxidase) Activity Assay Kit

Please read entire manual carefully before starting experiment.

Table of Contents

1. Key Features

Specification:

48T(46 samples)/96T(94 samples)

Measuring instrument:

Microplate reader (540-560 nm)

Detection range:

4.10-250.00 U/L

Sample type:

Animal tissue samples

2. Storage & Expiry

This product should be stored at -20°C in dark conditions for up to 12 months. For detailed storage instructions of individual kit components, please refer to Section 4. The expiration date is indicated on the outer label of the kit box.

3. Product Description

The Assay Genie Mitochondrial Complex IV Activity Assay Kit is designed to quantitatively measure the activity of mitochondrial Complex IV (cytochrome c oxidase) in biological samples.

Mitochondrial Complex IV is a key enzyme in the electron transport chain, responsible for oxidizing reduced cytochrome c (generated by Complex III) into its oxidized form while consuming molecular oxygen to produce water. Reduced cytochrome c exhibits a characteristic absorbance peak at 550 nm. As Complex IV converts reduced cytochrome c to its oxidized form, the absorbance at 550 nm decreases.

By monitoring the change in OD at 550 nm over time, the kit enables accurate quantification of Complex IV enzymatic activity.

If you want, I can also adapt this to match the exact tone and structure of your existing Assay Genie manuals.

This dual function kit includes validated Bradford Reagent to quantify total protein concentration for accurate sample normalization.

4. Kit Contents

No	Component Name	Size (48T)	Size (96T)	Storage
1	Extracting Solution A	50mL x 1vial	50mL x 2 vials	2-8℃,12 months (avoid light)
2	Extracting Solution B	50mL x 1vial	50mL x 2 vials	2-8℃,12 months (avoid light)
3	Inhibitor	1mL x 1vial	1mL x 2vials	2-8℃,12 months (avoid light)
4	Substrate	Powder x 2 vials	Powder x 4 vials	2-8℃,12 months (avoid light)
5	Stabilizer	Powder x 2 vials	Powder x 4 vials	2-8℃,12 months (avoid light)
6	Buffer Solution	13mL x 1vial	26mL x 1vial	2-8℃,12 months (avoid light)
7	Microplate	48 wells	96 wells	-
8	Plate Sealer	2 pieces	2 pieces	-
9	Bradford Reagent	2 mL	2 mL	RT

Note: *All reagents must be stored according to the specified conditions listed in the table above. Do not mix reagents from different kits, as this may compromise assay performance. For reagents provided in small volumes, centrifuge briefly before use to ensure complete recovery of the contents.*

Additional materials required:

- **Instruments:** Microplate reader (500-520 nm, optimum wavelength: 510 nm), Incubator (37℃)
- **Reagents:** Anhydrous ethanol, PBS (0.01M, pH7.4)

5. Important Notes

1. This assay kit is intended for Research Use Only. Assay Genie assumes no responsibility for any issues or legal liabilities arising from the use of this kit for clinical diagnostics or any other unauthorized purposes.
2. Please read the instructions carefully before beginning the assay. Ensure that all instruments are correctly calibrated. Strict adherence to the protocol is essential for accurate results.
3. Appropriate laboratory safety precautions must be followed, including the use of lab coats and latex gloves.
4. If the concentration of the target substance falls outside the detection range, please adjust the sample by performing further dilution or concentration as needed.
5. If your sample type is not listed in the instruction manual, we strongly recommend performing a preliminary test to confirm compatibility.
6. Experimental outcomes depend on multiple factors including reagent integrity, handling technique, and laboratory conditions. While Assay Genie guarantees the quality of our kits, we are not responsible for any loss of samples during use. We advise calculating sample requirements in advance and ensuring adequate sample volume is reserved before starting the assay.

6. Reagent Preparation

1. Equilibrate all reagents to 25℃ before use. Keep inhibitor, substrate and stabilizer on ice during use
2. **Preparation of substrate working solution:** Dissolve one vial of substrate with 4 mL of buffer solution, mix well. Aliquoted storage at-20°C for 1 month protected from light, and avoid repeated freeze/thaw cycles is advised.
3. **Preparation of stabilizer working solution:** Dissolve one vial of stabilizer with 200 µL of buffer solution, mix well. Storage at-20°C for 1 month protected from light, and avoid repeated freeze/thaw cycles is advised.
4. **Preparation of reaction working solution:** Before testing, please prepare sufficient reaction working solution according to the test wells. For example, prepare 2003 µL of reaction working solution (mix well 2000 µL of substrate working solution and 3 µL of stabilizer working solution). The reaction working solution should be prepared on spot. The reaction working solution should be placed at room temperature protected from light for 15 min before use. Storage at 4 h protected from light.

7. Sample preparation

A. Sample Preparation

Tissue samples

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. Homogenize100 mg tissue in 900 µL 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 11000×g for 10 min at 4°C, discard the supernatant and take the precipitate.

6. The precipitate was mixed with 200 µL of extraction solution B and 10 µL of inhibitor, sonicated for 1 min, centrifuged at 11000×g at 4°C for 10 min. Then take the supernatant for detection.

B. Dilution

Sample Type	Dilution factor
10% Rat liver tissue homogenate	5-20
10% Rat kidney tissue homogenate	5-20
10% Rat brain tissue homogenate	2-5
10%Rat lung tissue homogenate	2-5
10% Rat spleen tissue homogenate	2-5
10% Mouse liver tissue homogenate	5-20
10% Mouse kidney tissue homogenate	5-20
10% Mouse brain tissue homogenate	2-5
10% Mouse lung tissue homogenate	2-5
10% Mouse spleen tissue homogenate	2-5

Note: *The recommended diluent is double distilled water. For sample types not specified in the protocol, it is advised to perform a preliminary test to determine the appropriate dilution factor or contact our Tech Support Team at techsupport@assaygenie.com.*

7.1. Protein Quantification (Optional)

To quantify total protein levels, use the Bradford Reagent included in this kit. Visit Bradford Protein Assay Protocol to view the full protocol.

8. Assay Procedure

***Important**: It is recommended to limit each assay run to a maximum of five samples to ensure optimal performance and reliable data quality*

1. Blank well: Add 20 µL of extraction solution B to blank well. Sample well: Add 20 µL of sample to sample well. It is recommended
2. Add 140 µL of reaction working solution to each well and mix fully with microplate reader for 3 s.
3. Measure the OD value of each well at 550 nm with microplate reader at 10 s and 70s, respectively recorded as A1 and A2, ΔA = A1- A2.
   ***Note:** The average ΔA value of blank well should be within ± 0.005.*

9. Data Analysis

Tissue Sample

The amount of mitochondrial complex IV in 1 g tissue mitochondrial protein per 1 minute that oxidize 1 µmol of cytochrome C at room temperature is defined as 1 unit.

$$\text{mitochondrial complex IV activity (U/gprot)} = \frac{\Delta A_{550} \times V_1}{V_2 \times (\varepsilon \times d) \times T} \div C_{pr} \times f$$

Note:

ΔA550: ΔAsample– ΔAblank.

V1: The volume of the reaction system, 0.16 mL.

V2: The volume of the sample, 0.02 mL.

ε: Molar absorption coefficient, 0.0191 L/µmol/cm.

d: Optical path, 0.5 cm

T: The time of reaction, 1 min.

f: Dilution factor of sample before test.

Cpr: The concentration of protein in sample, gprot/L.

10. Typical Data

Sensitivity

The analytical sensitivity of the assay is 4.10 U/L. This value was determined by measuring the zero standard (blank) in 20 independent replicates, calculating the mean OD and adding two standard deviations. The corresponding concentration was then derived from the standard curve.

Recovery

Three sample concentrations (high, medium, and low) were tested in parallel, with six replicates per concentration. The average recovery rate across all concentrations was determined to be 102%.

	Sample 1	Sample 2	Sample 3
Expected Conc. (µmol/mL)	50.5	120.6	200.5
Observed Conc. (µmol/mL)	50.5	120.6	200.5
Recovery rate (%)	101	103	102

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 (µmol/mL)	50.5	120.6	200.5
% CV	4.5	4.0	3.9

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 (µmol/mL)	50.5	120.6	200.5
%CV	9.3	8.5	9.1

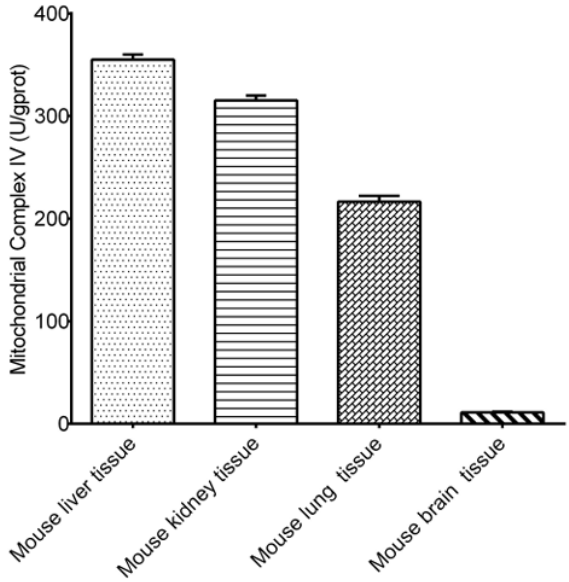
11. Example Analysis

For mouse liver tissue, take 20 μL of 10% mouse liver tissue mitochondrial supernatant, and carry the assay according to the operation steps. The results are as follows: the OD value of the sample A1 is 0.916, the OD value of the sample A2 is 0.760, the OD value of the blank A1 is 0.924, the OD value of the blank A2 is 0.920, the concentration of protein in sample is 3.54 gprot/L, and the calculation result is:

$$\text{mitochondrial complex IV (U/gprot)} = \frac{((0.916 - 0.760) - (0.924-0.920)) \times 0.16}{0.02 \times 0.0191 \times 0.5 \times 1} \div 3.54 \times 10$$

$$= 359.7 \text{ U/gprot}$$

Detect 10% mouse liver tissue homogenate (the concentration of mitochondrial protein is 3.54 gprot/L), 10% mouse kidney tissue homogenate (the concentration of mitochondrial protein is 6.49 gprot/L), 10% mouse lung tissue homogenate (the concentration of mitochondrial protein is 3.17 gprot/L) and 10% mouse brain tissue homogenate (the concentration of mitochondrial protein is 2.37 gprot/L) according to the protocol, the result is as follows:



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.