TECHNICAL MANUAL

Mitochondrial Complex I (NADH-CoQ Reductase) Activity Assay Kit

- **SKU CODE:** MAES0240
- **SIZE:** 48 Tests/96 Tests
- **DETECTION PRINCIPLE:** Assay Kit
- **RUO:** Research-Use-Only

Revised: 01/2026

1. Assay summary

- Reagent preparation
- Sample preparation
- Add sample and reagents to wells
- Incubate at 37°C for 3 min
- Add reaction working solution
- Measure absorbance at 340 nm

2. Intended use

This kit can measure mitochondrial complex I (NADH-CoQ Reductase) activity in animal tissue samples.

3. Detection principle

Mitochondrial complex I catalyzes the reaction of NADH with ubiquinone substrate to generate NAD+ and reduced ubiquinone. The activity of NADH is reflected by measuring the absorbance decline rate at 340 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	25 mL × 1 vial	50 mL × 1 vial	-20°C, 12 months
Reagent 3	Protease Inhibitor	0.4 mL × 1 vial	0.4 mL × 2 vials	-20°C, 12 months, shading light
Reagent 4	Buffer Solution	15 mL × 1 vial	15 mL × 2 vials	-20°C, 12 months, shading light
Reagent 5	Substrate A	Powder × 1 vial	Powder × 2 vials	-20°C, 12 months, shading light
Reagent 6	Substrate B	Powder × 1 vial	Powder × 1 vial	-20°C, 12 months, shading light

Item	Component	Size 1(48 T)	Size 2(96 T)	Storage
Reagent 7	Inhibitor	1.5 mL × 1 vial	1.5 mL × 2 vials	-20°C, 12 months, shading light
Reagent 8	Negative Reagent	1.5 mL × 1 vial	1.5 mL × 2 vials	-20°C, 12 months, shading light
	UV Microplate	96 wells	96 wells	No requirement
	Plate Sealer	2 pieces	2 pieces	

5. Materials prepared by users

Instruments:

Centrifuge, 37°C incubator, Microplate reader (340 nm)

Reagents:

Anhydrous ethanol (AR)

6. Reagent preparation

1. Equilibrate all reagents to room temperature before use.
2. Preparation of substrate A solution: Dissolve one vial of substrate A with 150 µL of double distilled water and mix well to dissolve. Store at -20°C for 7 days protected from light.
3. Preparation of substrate A working solution: Before testing, prepare sufficient working solution according to the sample wells. For example, prepare 1012 µL of substrate A working solution (mix well 12 µL of substrate A solution and 1000 µL of buffer solution). Store at 2-8°C for 12 hours protected from light.
4. Preparation of substrate B working solution: Dissolve one vial of substrate B with 4 mL of anhydrous ethanol and shake until it turns yellowish clear liquid. Store at 2-8°C for 48 hours protected from light. Aliquots can be stored at -20°C for 7 days protected from light.
5. Preparation of reaction working solution: Before testing, prepare sufficient reaction working solution according to the test wells. For example, prepare 250 µL of reaction working solution (mix well 5 µL of substrate B working solution and 245 µL of substrate A working solution). The reaction working solution should be prepared immediately before use and protected from light, keep on ice during use within 1 hour.

7. Sample preparation

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 µL extraction solution A and 10 µL protease inhibitor with a dounce homogenizer at 4°C.

4) Centrifuge at 600×g at 4°C for 5 min, discard the precipitate and take the supernatant.

5) Then centrifuge at 15000×g for 10 min at 4°C, discard the supernatant and take the precipitate.

6) Mix the precipitate with 200 µL of extraction solution B and 2 µL of protease inhibitor, sonicate for 1 min, centrifuge at 15000×g at 4°C for 10 min. Then take the supernatant for detection.

7) Meanwhile, determine the protein concentration of supernatant (MAES0177).

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

10% Rat muscle tissue homogenate: 1-2

10% Rat lung tissue homogenate: 4-8

10% Mouse liver tissue homogenate: 1-2

10% Mouse heart tissue homogenate: 4-8

10% Rat heart tissue homogenate: 1-2

10% Rat liver tissue homogenate: 1-2

10% Rat kidney tissue homogenate: 2-4

10% Porcine heart tissue homogenate: 1-2

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. During reagent preparation, ensure that the prepared substrate B working solution is completely dissolved. It is recommended to extend the oscillation time and transfer the reagent to an EP tube to check whether the reagent is completely dissolved to clarity.
2. During sample measurement, if the OD value decreases by more than 0.3 within 3 min, the sample should be diluted to ensure that the sample measurement is within the interval of uniform reaction speed. If necessary, the OD value can be measured every minute to observe its dynamic changes.
3. It is recommended to use fresh samples for detection.
4. It is better to measure no more than 8 sample wells at the same time.

9. Operating steps

1. Control well: Add 20 μL of sample to the corresponding wells. Sample well: Add 20 μL of sample to the corresponding wells.
2. Control well: Add 20 μL of negative reagent to the corresponding wells. Sample well: Add 20 μL of inhibitor to the corresponding wells.
3. Mix fully and incubate at 37°C for 3 min.
4. Add 200 μL of reaction working solution to each well.
5. Measure the OD value of each well at 340 nm with microplate reader, recorded as A1. 3 min later, measure the OD value of each well at 340 nm with microplate reader, recorded as A2, ΔA = A1 - A2.
   Note: The control wells measure the total enzyme activity, and the sample wells measure the non-specific enzyme activity. After adding the reaction working solution, record the OD value once every minute for 3 min, observe the change of OD value within 3 min to ensure whether there is a constant rate of decline.

10. Calculation

For tissue sample:

Definition: The amount of mitochondrial complex I in 1 g tissue mitochondria protein per 1 minute that catalyzes the decomposition of 1 μmol NADH at 37°C is defined as 1 unit.

mitochondrial complex I activity

$$(U/gprot) = (\Delta A\_Control - \Delta A\_Sample) / (6600 \times 0.7) \times V_1 \div T \div V_2 \div C_{pr} \times f \times 10^6$$

[Note]

ΔA_Control: The change OD value of control (A_1 - A_2).

ΔA_Sample: The change OD value of sample (A_1 - A_2).

6600: The molar extinction coefficient of NADH, L/(mol•cm)

0.7: Optical path, cm

V_1: The volume of the reaction system, 0.24 mL.

V_2: The volume of the sample, 0.02 mL.

T: The time of reaction, 3 min.

f: Dilution factor of sample before test.

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

10^6: 1 mol = 10^6 μmol.

11. Appendix

Performance Characteristics

Intra-assay Precision

Three rat lung 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)	32.50	105.00	197.00
%CV	1.0	0.7	0.7

Inter-assay Precision

Three rat lung 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)	32.50	105.00	197.00
%CV	8.2	8.6	8.4

Recovery

Three samples of high concentration, middle concentration and low concentration were tested with 6 replicates of each concentration to obtain an average recovery rate of 102%.

Sample	Expected Conc. (U/L)	Observed Conc. (U/L)	Recovery rate (%)
Sample 1	56.8	58.5	103
Sample 2	135	132.3	98
Sample 3	188	197.4	105

Sensitivity

The analytical sensitivity of the assay is 4.33 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.

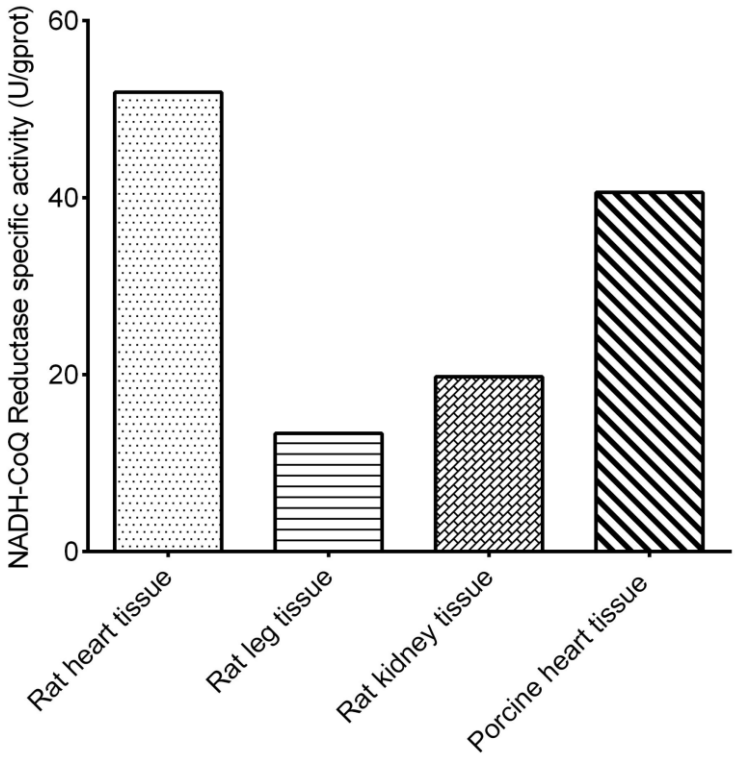
Example Analysis

For 20% rat heart tissue homogenate, diluted 2 times, take 20 μL and carry out the assay according to the operation steps. The results are as follows:

The A_1 of the control is 0.597, the A_1 of the sample is 0.722. After 3 minutes, the A_2 of the control is 0.433, the A_2 of the sample is 0.711, $\Delta A\_Control = A_1 - A_2 = 0.597 - 0.433 = 0.164$, $\Delta A\_Sample = A_1 - A_2 = 0.722 - 0.711 = 0.011$, the concentration of mitochondria protein in sample is 3.75 gprot/L, and the calculation result is:

mitochondrial complex I activity (U/gprot) = $(0.164 - 0.011) / (6600 \times 0.7) \times 0.24 \div 3 \div 0.02 \div 3.75 \times 2 \times 10^6 = 70.65$ U/gprot

Detected 20% rat heart tissue homogenate (the concentration of mitochondria protein is 3.97 gprot/L, diluted 2 times), 20% rat leg tissue homogenate (the concentration of mitochondria protein is 2.04 gprot/L, diluted 2 times), 20% rat kidney tissue homogenate (the concentration of mitochondria protein is 7.88 gprot/L, diluted 4 times) and 20% Porcine heart tissue homogenate (the concentration of protein is 1.54 gprot/L) according to the protocol, the result is as follows:



12. 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:

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.