TECHNICAL MANUAL

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

- **SKU CODE:** MAES0464
- **SIZE:** 48 Tests/96 Tests
- **DETECTION PRINCIPLE:** Assay Kit
- **RUO:** Research-Use-Only

Revised: 06/2026

1. Assay summary

- Reagent preparation
- Sample preparation
- Add sample
- Measure the OD value

2. Intended use

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

3. Detection principle

Mitochondrial complex I catalyzes the reaction of NADH with the ubiquinone substrate to generate NAD^+ and reduced ubiquinone. The activity of mitochondrial complex I can be quantified by measuring the change in OD value at 340 nm.

4. Kit components & storage

Item	Component	Size 1 (48 T)	Size 2 (96 T)	Storage
Reagent 1	Extraction Solution	25 mL × 1 vial	50 mL × 1 vial	-20 °C, 12 months
Reagent 2	Protease Inhibitor	0.8 mL × 1 vial	0.8 mL × 2 vials	-20 °C, 12 months, protect from light
Reagent 3	Buffer Solution	15 mL × 1 vial	15 mL × 2 vials	-20 °C, 12 months, protect from light
Reagent 4	Substrate A	Powder × 1 vial	Powder × 2 vials	-20 °C, 12 months, protect from light
Reagent 5	Substrate B	Powder × 1 vial	Powder × 2 vials	-20 °C, 12 months, protect from light
	UV Microplate	96 wells		No requirement
	Plate Sealer	2 pieces		

Item	Component	Size 1 (48 T)	Size 2 (96 T)	Storage
	Sample Layout Sheet	1 piece		

5. Materials prepared by users

Instruments:

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

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. Store at -20 °C for 7 days protected from light.
3. **Preparation of substrate A working solution:** Before testing, prepare sufficient substrate A working solution according to the number of test wells. For example, prepare 505 µL by mixing 5 µL of substrate A solution with 500 µL of Buffer Solution. Store at 2-8 °C for 12 h 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 into a yellowish clear liquid. Store at 2-8 °C for 48 h protected from light; for aliquoted storage, keep 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 number of test wells. For example, prepare 300 µL by mixing 5 µL of substrate B working solution with 295 µL of substrate A working solution. The reaction working solution should be prepared on the spot and kept on ice for use within 1 h.

7. Sample preparation

Sample preparation:

Cell (adherent or suspension) samples:

1. Harvest the number of cells needed for each assay (initial recommendation 1×10^6 cells).
2. Wash cells with PBS (0.01 M, pH 7.4).
3. Homogenize 1×10^6 cells in 200 µL extraction solution and 4 µL protease inhibitor with an ultrasonic cell disruptor (4 °C, 200 W, 5 s/time, 10 s interval, repeat 15 times).
4. Centrifuge at 10,000×g for 3 min at 4 °C to remove insoluble material. Collect supernatant and keep it on ice for detection.
5. Meanwhile, determine the protein concentration of supernatant (MAES0177).

Dilution of sample:

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

- Jurkat cell: 1

- CHO cell: 1

- HL-60 cell: 1

- Hela cell: 1

- 293T cell: 1

- Molt-4 cell: 1

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

8. Operating steps

Key points of the assay:

1. During reagent preparation, ensure the prepared substrate B working solution is completely dissolved; it is recommended to extend the oscillation or ultrasound time.
2. During sample measurement, if the OD value decreases by more than 0.3 within 3 min, the sample should be diluted.
3. It is recommended to use fresh sample for detection.
4. It is better to measure no more than 8 sample wells at the same time.

Steps:

1. Sample well: Add 20 µL of sample to the corresponding wells.
2. Add 200 µL of reaction working solution to each well.

3. Measure the OD value of each well at 340 nm with a microplate reader, recorded as A1. Incubate at 37 °C for 5 min, then measure the OD value of each well at 340 nm again, recorded as A2. ΔA = A1 - A2.

9. Calculation

The sample:

Definition: The amount of mitochondrial complex I in 1 g mitochondrial protein that hydrolyzes the substrate to produce 1 μmol product per minute at 37 °C is defined as 1 unit.

Mitochondrial complex I activity (U/gprot) = $\Delta A_{Sample} \div (6600 \times 0.7) \times V1 \div T \div V2 \div Cpr \times f \times 10^6$

[Note]

ΔA_{Sample}: The change in OD value of the sample (A1 - A2).

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

0.7: Optical path, cm.

V1: The volume of the reaction system, 0.22 mL.

V2: The volume of the sample, 0.02 mL.

T: The time of reaction, 5 min.

f: Dilution factor of sample before test.

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

10^6: 1 mol = 10^6 μmol.

10. Appendix I Performance Characteristics

Parameter:

Intra-assay Precision

Three Molt-4 cell 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)	30.00	60.00	90.00
%CV	3.0	3.8	4.0

Inter-assay Precision

Three Molt-4 cell 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)	30.00	60.00	90.00
%CV	5.0	6.5	8.0

Recovery

Three samples of high, middle, and low concentration were tested with each concentration run 6 times in parallel to obtain an average recovery rate of 99%.

Standard 1	Standard 2	Standard 3
Expected Conc. (U/L): 20	Expected Conc. (U/L): 50	Expected Conc. (U/L): 100
Observed Conc. (U/L): 19.0	Observed Conc. (U/L): 48.5	Observed Conc. (U/L): 105.0
Recovery rate (%): 95	Recovery rate (%): 97	Recovery rate (%): 105

Sensitivity

The analytical sensitivity of the assay is 2.85 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. The detection range of the assay is 2.85-119.04 U/L.

11. Appendix II Example Analysis

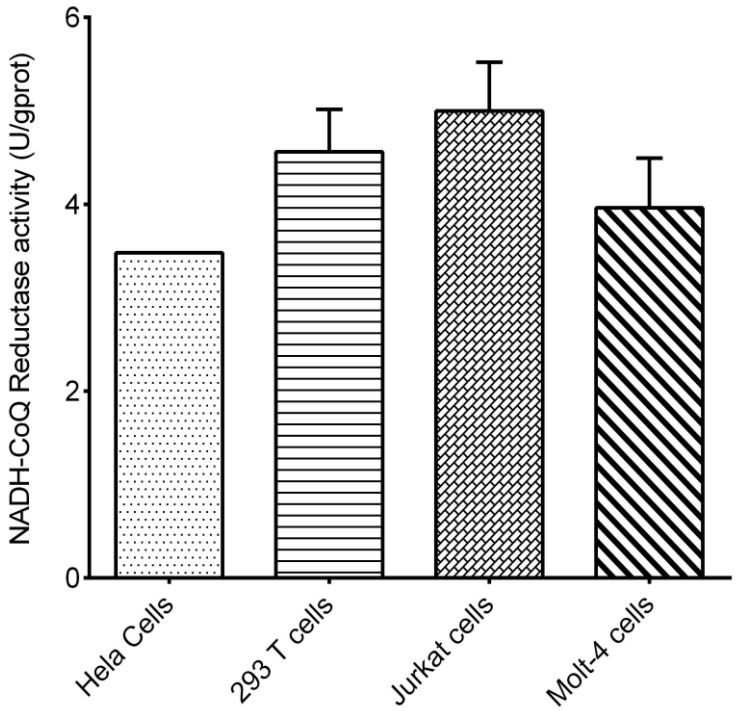
Example analysis:

Take 20 μL of Hela cell for detection and carry out the assay according to the operating steps. The results are as follows:

The A1 of the sample is 0.672. After 5 minutes, the A2 of the sample is 0.66, ΔA_Sample = A1 - A2 = 0.672 - 0.66 = 0.012, the concentration of mitochondrial protein in the sample is 1.64 gprot/L, and the calculation result is:

Mitochondrial complex I activity (U/gprot) = 0.012 ÷ (6600 × 0.7) × 0.22 ÷ 5 ÷ 0.02 ÷ 1.64 × 10^6 = 3.48 U/gprot

Detect Hela (1×10^6, protein concentration 1.64 gprot/L), 293T cell (1×10^6, protein concentration 1.04 gprot/L), Jurkat cell (1×10^6, protein concentration 1.02 gprot/L) and Molt-4 cell (1×10^6, protein concentration 0.63 gprot/L, diluted 10 times) according to the protocol. The results are 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. 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.

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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.