TECHNICAL MANUAL

Mitochondrial Respiratory Chain Complex (F0F1-ATPase /ATP Synthase) Activity Assay Kit

- **SKU CODE:** MAES0250
- **SIZE:** 48 Tests
- **DETECTION PRINCIPLE:** Assay Kit
- **RUO:** Research-Use-Only

Revised: 01/2026

1. Assay summary

- Sample preparation
- Add specific working solution or water
- Add sample
- Incubate at 37°C
- Add enzyme working solution
- Add reaction working solution
- Measure OD values

2. Intended use

This kit can measure mitochondrial complex V (F0F1-ATPase/ATP Synthase) activity in animal tissue samples.

3. Detection principle

Mitochondrial complex V is also known as F0F1-ATP synthase. ATP is hydrolyzed by F0F1-ATP synthase to produce ADP, and ADP converts NADH into oxidized coenzyme I (NAD+) after enzymatic conversion reaction. Therefore, the activity of mitochondrial complex V 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 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	0.8 mL × 1 vial	0.8 mL × 2 vials	-20°C, 12 months, protect from light
Reagent 4	Buffer Solution	15 mL × 1 vial	30 mL × 1 vial	-20°C, 12 months

Item	Component	Size 1(48 T)	Size 2(96 T)	Storage
Reagent 5	Substrate A	Liquid × 1 vial	Liquid × 2 vials	-20°C, 12 months, protect from light
Reagent 6	Substrate B	Powder × 1 vial	Powder × 2 vials	-20°C, 12 months, protect from light
Reagent 7	Substrate C	Powder × 1 vial	Powder × 2 vials	-20°C, 12 months, protect from light
Reagent 8	Substrate D	Powder × 2 vials	Powder × 4 vials	-20°C, 12 months, protect from light
Reagent 9	Inhibitor	0.05 mL × 1 vial	0.1 mL × 1 vial	-20°C, 12 months, protect from 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 (330-350 nm, optimum wavelength: 340 nm)

6. Reagent preparation

1. Equilibrate all reagents to room temperature before use.
2. **Preparation of Substrate B working solution:** Dissolve one vial of Substrate B with 300 µL of double distilled water, mix well. Aliquot and store at -20°C for 7 days protected from light.
3. **Preparation of Substrate C working solution:** Dissolve one vial of Substrate C with 300 µL of double distilled water, mix well. Aliquot and store at -20°C for 7 days protected from light.
4. **Preparation of reaction working solution:** Before testing, prepare sufficient reaction working solution according to the test wells. For example, prepare 525 µL of reaction working solution (mix well 500 µL of buffer solution, 5 µL of Substrate A, 10 µL of Substrate B working solution, 10 µL of Substrate C working solution). The prepared solution can be stored at 2-8°C for 8 h protected from light.

5. **Preparation of enzyme working solution:** Dissolve one vial of Substrate D with 0.6 mL of double distilled water, mix well. Store at 2-8°C for 3 days protected from light.
6. **Preparation of specific working solution:** Before testing, prepare sufficient specific working solution according to the test wells. For example, prepare 505 µL of specific working solution (mix well 5 µL of inhibitor and 500 µL of double distilled water). The specific working solution should be prepared on spot. The remaining inhibitor can be stored at -20°C for 1 week.

7. 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 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 15,000×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 protease inhibitor, sonicated for 5 min, centrifuged at 15,000×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):

8. Sample dilution recommendations

Sample type	Dilution factor
10% Mouse liver tissue homogenate	2-6
10% Rat liver tissue homogenate	1-4
10% Mouse kidney tissue homogenate	2-6
10% Rat muscle tissue homogenate	1-3

9. The key points of the assay

1. During reagent preparation, ensure that the powder is completely dissolved in the reaction working solution after preparation.
2. Detection is started at about 10 s after adding reaction working solution.
3. For sample detection, if the A1 of the sample well and the control well is lower than 0.7, or the change OD value (ΔA) of the sample well and the control well for 4 min exceeds 0.3, the sample should be diluted.

10. Operating steps

1. Control well: Add 10 μL of specific working solution to the corresponding wells. Sample well: Add 10 μL of double distilled water to the corresponding wells.
2. Add 20 μL of sample to each well.
3. Mix fully with microplate reader for 3 s and incubate at 37°C for 4 min.
4. Add 20 μL of enzyme working solution to each well.
5. Add 180 μL of reaction working solution to each well.
6. Measure the OD value of each well at 340 nm with microplate reader, recorded as A1. After 4 min, measure the OD value of each well at 340 nm with microplate reader, recorded as A2. ΔA = A1 - A2.

11. Calculation

For tissue samples:

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

Mitochondrial complex V activity (U/g_{prot}) = (ΔA_sample - ΔA_control) / (6220 × 0.65) × 0.23 ÷ t ÷ 0.02 ÷ C_{pr} × f × 10^6

Where:

ΔA_sample: The change OD value of sample well, A_1-A_2

ΔA_control: The change OD value of control well, A_1-A_2

6220: Molar absorption coefficient, L/(μmol*cm)

0.65: Optical path, cm

0.23: The volume of the reaction system, mL

0.02: The volume of the sample, mL

t: The time of reaction, 4 min

f: Dilution factor of sample before test

C_{pr}: The concentration of protein in sample, g_{prot}/L

10^6: 1 mol = 10^6 μmol

12. Appendix I Performance Characteristics

Parameter:

Intra-assay Precision

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

Intra-assay Precision Results

Parameters	Sample 1	Sample 2	Sample 3
Mean (U/L)	21.50	38.50	65.60
%CV	5.8	5.6	5.4

Inter-assay Precision

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

Inter-assay Precision Results

Parameters	Sample 1	Sample 2	Sample 3
Mean (U/L)	21.50	38.50	65.60
%CV	6.4	6.3	6.8

Recovery

Three samples of high, middle, and low concentrations were tested with 6 replicates each to obtain an average recovery rate of 101%.

Recovery Results

Sample	Expected Conc. (U/L)	Observed Conc. (U/L)	Recovery rate (%)
Sample 1	25	25.8	103
Sample 2	32.4	32.1	99
Sample 3	70.2	70.9	101

Sensitivity

The analytical sensitivity of the assay is 3.55 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.

13. Appendix II Example Analysis

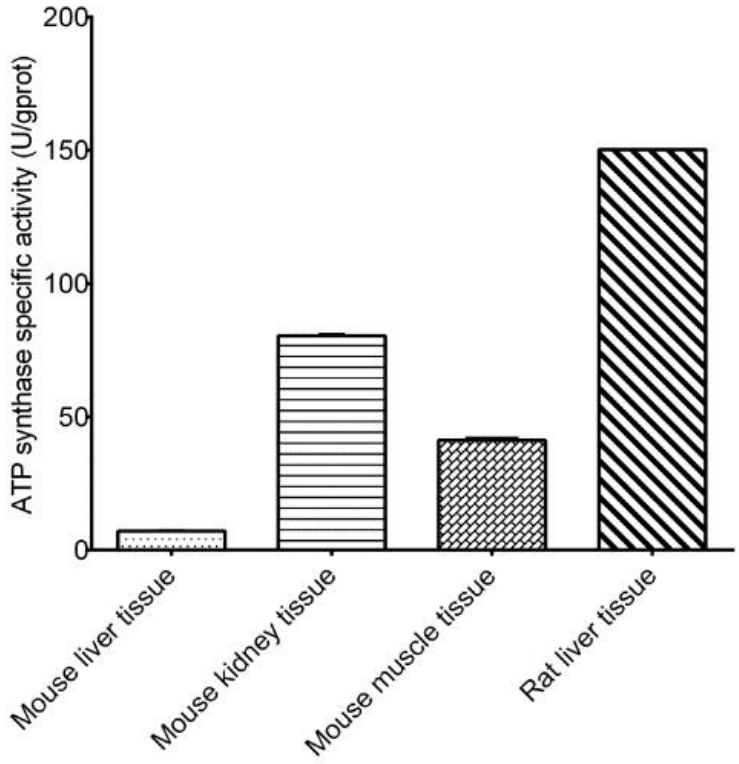
Example analysis:

For 10% mouse liver tissue mitochondria supernatant, diluted 4 times, carry out the assay according to the operation steps. The results are as follows:

The A_1 of the sample well is 0.900, the A_2 of the sample well is 0.426, ΔA_sample = 0.900 - 0.426 = 0.474. The A_1 of control well is 0.914, the A_2 of control well is 0.471, ΔA_control = 0.914 - 0.471 = 0.443, the concentration of mitochondria protein in sample is 12.14 g_{prot}/L, and the calculation result is:

Mitochondrial complex V activity (U/g_{prot}) = (0.474 - 0.443) / (6220 × 0.65) × 0.23 ÷ 4 ÷ 0.02 ÷ 12.14 × 4 × 10^6 = 7.26 U/g_{prot}

Detect 10% mouse liver tissue homogenate (the concentration of mitochondria protein is 12.14 g_{prot}/L, diluted 4 times), 10% mouse kidney tissue homogenate (the concentration of mitochondria protein is 12.33 g_{prot}/L, diluted 4 times), 10% mouse muscle tissue homogenate (the concentration of mitochondria protein is 1.86 g_{prot}/L, diluted 4 times) and 10% rat liver tissue homogenate (the concentration of mitochondria protein is 7.50 g_{prot}/L, diluted 4 times) according to the protocol, the results are as follows:



14. 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!

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.