TECHNICAL MANUAL

ATP Colorimetric Assay Kit (Enzyme)

- **SKU CODE:** MAES0440
- **SIZE:** 96 Tests
- **DETECTION PRINCIPLE:** Assay Kit
- **RUO:** Research-Use-Only

Revised: 01/2026

1. Assay summary

- Reagent preparation
- Standard curve preparation
- Sample preparation
- Add standards and samples
- Measure the OD value

2. Intended use

This kit can be used to measure ATP content in animal tissue samples.

3. Detection principle

Adenosine Triphosphate (ATP) is a high-energy phosphate compound, which is the most direct energy source in the body, and its content is directly related to the state of energy metabolism in the body. Hexokinase (HK) catalyzes the generation of glucose and ATP into glucose-6-phosphate, and glucose-6-phosphate dehydrogenase (G6PD) further catalyzes the dehydrogenation of glucose-6-phosphate into NADPH. NADPH can react with the color developer, and the product has a characteristic absorption peak at 460 nm to determine the ATP content.

4. Kit components & storage

Item	Component	Size (96 T)	Storage
Reagent 1	Extraction Solution	50 mL × 2 vials	-20°C, 12 months
Reagent 2	Enzyme diluent	8 mL × 1 vial	-20°C, 12 months
Reagent 3	Enzyme Reagent A	Powder × 1 vial	-20°C, 12 months, shading light
Reagent 4	Enzyme Reagent B	0.2 mL × 1 vial	-20°C, 12 months, shading light
Reagent 5	Enzyme Reagent C	0.2 mL × 1 vial	-20°C, 12 months, shading light
Reagent 6	Chromogenic Agent	6 mL × 1 vial	-20°C, 12 months, shading light

Item	Component	Size (96 T)	Storage
Reagent 7	100 μmol/L Standard Solution	1.5 mL × 2 vials	-20°C, 12 months
	Microplate	96 wells	No requirement
	Plate Sealer	2 pieces	

5. Materials prepared by users

Instruments:

Microplate reader (450-470 nm, optimum wavelength: 460 nm), Incubator (37°C), Water bath

Reagents:

PBS (0.01 M, pH 7.4)

6. Reagent preparation

1. Keep enzyme reagent A on ice during use. Equilibrate other reagents to 25°C before use.
2. **Preparation of enzyme A application solution:** Dissolve one vial of enzyme reagent A with 1 mL of double distilled water, mix well to dissolve. Store at -20°C for a month protected from light.
3. **Preparation of enzyme A working solution:** Before testing, prepare sufficient enzyme A working solution according to the test wells. For example, prepare 75 μL of enzyme A working solution (mix well 5 μL of enzyme A application solution and 70 μL of double distilled water). Store at -20°C and use within the same day.
4. **Preparation of enzyme B working solution:** Dissolve one vial of enzyme reagent B with 2.3 mL of enzyme diluent, mix well to dissolve. Store at -20°C for a month protected from light.
5. **Preparation of enzyme C working solution:** Dissolve one vial of enzyme reagent C with 3.3 mL of enzyme diluent, mix well to dissolve. Store at -20°C for a month protected from light.
6. **Preparation of 40 μmol/L standard solution:** Before testing, prepare sufficient 40 μmol/L standard solution. For example, prepare 1000 μL of 40 μmol/L standard

solution (mix well 400 µL of 100 µmol/L standard solution and 600 µL of extraction solution). Store at -20°C for a month.

7. **Preparation of standard curve:** Always prepare a fresh set of standards. Discard working standard dilutions after use. Dilute 40 µmol/L standard solution with extraction solution to a serial concentration. The recommended dilution gradient is as follows: 0, 5, 10, 15, 20, 25, 30, 40 µmol/L.

7. Sample preparation

Animal tissue sample:

1. Harvest the amount of tissue needed for each assay (initial recommendation 50 mg).

2. Wash tissue in cold PBS (0.01 M, pH 7.4).

3. Homogenize 50 mg tissue in 450 µL extracting solution with a dounce homogenizer at 4°C.

4. Incubate in boiling water bath for 3 min, cool the tubes to 25°C with running water.

5. Centrifuge at 10000×g for 10 min at 4°C to remove insoluble material. Collect supernatant and keep it on ice for detection.

Dilution of sample:

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

Sample type - Dilution factor

10% Mouse heart tissue homogenate - 1

10% Mouse kidney tissue homogenate - 1

10% Mouse muscle tissue homogenate - 1

10% Mouse liver tissue homogenate - 1

10% Mouse brain tissue homogenate - 1

10% Fish muscle tissue homogenate - 1

Note: The diluent is extraction solution. For the dilution of other sample types, please do pretest to confirm the dilution factor.

8. The key points of the assay

The fresh samples should be used.

9. Operating steps

1. Standard well: Add 100 µL of different concentrations solution to standard well. Sample well: Add 100 µL of sample to sample well. Control well: Add 100 µL of sample to control well.
2. Add 50 µL of enzyme A working solution to each well.
3. Add 50 µL of enzyme C working solution to standard and sample wells. Add 50 µL of enzyme B working solution to control wells.
4. Add 50 µL of chromogenic agent to each well.
5. Mix fully with microplate reader for 5 s. Incubate at 37°C for 5 min, measure the OD value of each well at 460 nm with microplate reader.

10. Calculation

The standard curve:

1. Average the duplicate reading for each standard.

2. Subtract the mean OD value of the blank (Standard # ①) from all standard readings. This is the absolute OD value.

3. Plot the standard curve by using absolute OD value of standard and correspondent concentration as y-axis and x-axis respectively. Create the standard curve (y = ax + b) with graph software (or EXCEL).

The sample:

Tissue sample:

ATP content (µmol/kg wet weight) = (ΔA - b) / a × f ÷ m / V

[Note]

ΔA: $\Delta A = OD_{sample} - OD_{control}$

m: The wet weight of sample, g.

V: The volume of extraction solution in the preparation step, mL.

f: Dilution factor of sample before test.

Intra-assay Precision

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

Inter-assay Precision

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

Recovery

Take three samples of high concentration, middle concentration and low concentration to test the samples of each concentration for 6 times parallelly to get the average recovery rate of 104.4%.

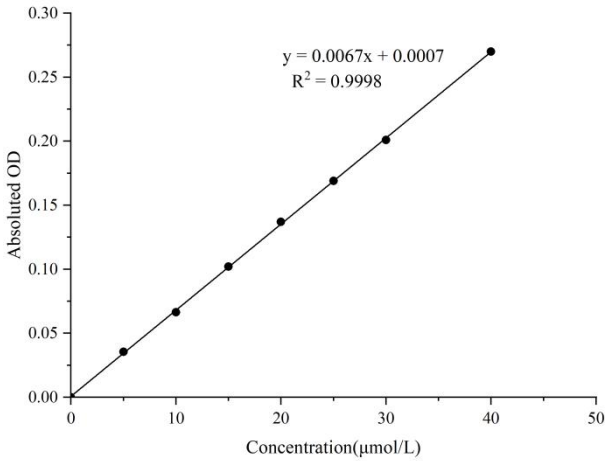
Sensitivity

The analytical sensitivity of the assay is 0.72 μmol/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.

Standard curve

(It doesn't need to prepare the standard curve for this kit and the provided standard curve is for reference only)

As the OD value of the standard curve may vary according to the conditions of the actual assay performance (e.g. operator, pipetting technique or temperature effects), so the standard curve and data are provided as below for reference only.



11. Appendix II Example Analysis

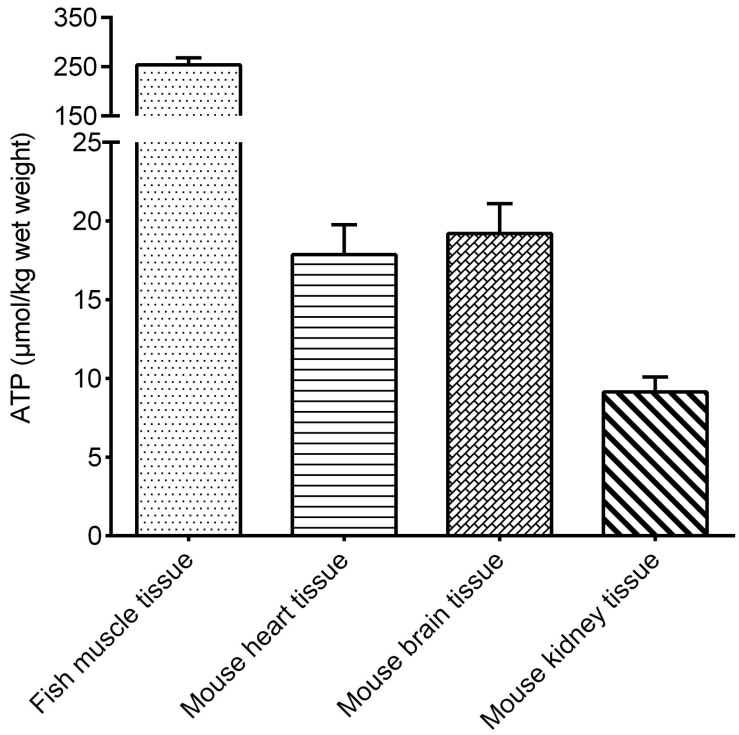
Example analysis:

Take 100 μL of 10% fish muscle tissue homogenate and carry the assay according to the operation steps. The results are as follows:

Standard curve: y = 0.0067 x + 0.0007. The average OD value of the sample well is 0.432, the average OD value of the control well is 0.243, ΔA = 0.432 - 0.243 = 0.189, and the calculation result is:

ATP content (μmol/kg wet weight) = (0.189 - 0.0007) ÷ 0.0067 ÷ 0.1 × 0.9 = 252.94 μmol/kg wet weight

Detect 10% fish muscle tissue homogenate, 10% mouse heart tissue homogenate, 10% mouse brain tissue homogenate and 10% mouse kidney tissue homogenate 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.

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.