ATP Assay Kit

Catalog No.: abx295152

Size: 96 tests

Detection Range: 0.72 µmol/L - 40 µmol/L

Sensitivity: 0.72 µmol/L

Storage: Store all components at -20°C. Store the Chromogenic Reagent and Enzyme Reagents in the dark.

Application: For the detection and quantification of ATP content in animal tissue homogenates.

For Reference Only

Principle of the Assay

Abbexa's ATP Assay Kit is a quick, convenient, and sensitive method for measuring and calculating ATP concentration. In the presence of hexokinase (HK), ATP and glucose react to generate glucose-6-phosphate. Glucose-6-phosphate dehydrogenase (G6PD) further catalyzes the dehydrogenation of glucose-6-phosphate to produce NADPH. NADPH reacts with a chromogenic reagent to produce a colored compound, with an absorbance maximum at 460 nm. The intensity of the color is directly proportional to the ATP concentration, which can then be calculated.

Kit components

1. 96-well microplate
2. Extraction Solution: 2 × 50 ml
3. Enzyme Diluent: 8 ml
4. Enzyme Reagent A: 1 vial
5. Enzyme Reagent B: 0.2 ml
6. Enzyme Reagent C: 0.2 ml
7. Chromogenic Reagent: 6 ml
8. Standard Solution (100 µmol/L): 2 × 1.5 ml
9. Plate sealer: 2

Materials required but not provided

1. Microplate reader (460 nm)
2. Distilled water
3. PBS (0.01 M, pH 7.4)
4. Pipette and pipette tips
5. Vials/tubes
6. Water bath
7. Centrifuge
8. Vortex mixer
9. Incubator

Protocol

A. Preparation of samples and reagents

1. Reagents

- **Enzyme A Application Solution:** Reconstitute 1 vial of Enzyme Reagent A with 1 ml of double-distilled water. Mix thoroughly, until fully dissolved. Store at -20°C for up to 1 month, protected from light.
- **Enzyme A Working Solution:** Prepare enough Enzyme A Working Solution for the wells tested. For example, prepare 75 µl of Enzyme A Working Solution by mixing 5 µl of Enzyme A Application Solution with 70 µl of double-distilled water. Store at -20°C and use on the same day.
- **Enzyme B Working Solution:** Reconstitute 1 vial of Enzyme Reagent B with 2.3 ml of Enzyme Diluent. Mix thoroughly, until fully dissolved. Store at -20°C for up to 1 month, protected from light.
- **Enzyme C Working Solution:** Reconstitute 1 vial of Enzyme Reagent C with 3.3 ml of Enzyme Diluent. Mix thoroughly, until fully dissolved. Store at -20°C for up to 1 month, protected from light.
- **40 µmol/L Standard Solution:** Prepare enough 40 µmol/L Standard Solution for the wells tested. For example, prepare 1000 µl of 40 µmol/L Standard Solution by mixing 400 µl of 100 µmol/L Standard Solution with 600 µl of Extraction Solution. Store at -20°C for up to 1 month.
- **Standards:** Label 7 tubes with 5 µmol/L, 10 µmol/L, 15 µmol/L, 20 µmol/L, 25 µmol/L, 30 µmol/L, and 40 µmol/L. Prepare these dilutions according to the volumes in the following table:

Standard Dilution (µmol/L)	5	10	15	20	25	30	40
40 µmol/L Standard (µl)	100	200	300	400	500	600	800
Extraction solution (µl)	700	600	500	400	300	200	0

For the blank, use pure Extraction Solution. The volume of each standard dilution will be 800 µl.

Note:

- Keep Enzyme Reagent A on ice during use.
- Allow all other reagents to equilibrate to room temperature before use.

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2. Samples

Isolate the test samples soon after collecting and analyze immediately or aliquot and store at -20°C or -80°C for long-term storage. Avoid multiple freeze-thaw cycles.

The following sample preparation methods are intended as a guide and may be adjusted as required depending on the specific samples used.

- **Tissue homogenates:** Carefully weigh at least 50 mg of tissue. Wash the tissue with cold PBS (0.01 M, pH 7.4). Per 50 mg of tissue, add 450 µl of Extraction Solution. Homogenize manually using a mechanical homogenizer at 4°C. Incubate in a water bath at 100°C for 3 minutes, then cool to room temperature under running water. Centrifuge at 10,000 × g for 10 minutes at 4°C. Carefully collect the supernatant. Keep on ice and assay immediately, or aliquot and store at -80°C for up to 6 months.

It is recommended to carry out a preliminary experiment to determine the optimal dilution factor of samples before carrying out the formal experiment. Dilute samples into different concentrations with Extraction Solution, then carry out the assay procedure. 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

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Note:

- Fresh samples or recently obtained samples are recommended to prevent degradation and denaturalization that may lead to erroneous results.
- Lysis buffers may interfere with the performance of this kit. It is recommended to use mechanical lysis methods only for tissue homogenates.

B. Assay Procedure

Preheat the incubator, and ensure it has reached a stable temperature before use.

1. Mark positions on the 96-well microplate for each standard, blank, sample, and control. *Each sample requires a corresponding control. It is strongly recommended to test all wells in duplicate*.
2. Add 100 µl of each standard dilution to the standard wells.
3. Add 100 µl of Extraction Solution to the blank wells.

4. Add 100 µl of sample to the sample wells.
5. Add 100 µl of sample to the corresponding control wells.
6. Add 50 µl of Enzyme A Working Solution to the standard, sample, and control wells.
7. Add 50 µl of Enzyme C Working Solution to the standard and sample wells. Add 50 µl of Enzyme B Working Solution to the control wells.
8. Add 50 µl of Chromogenic Reagent to all wells.
9. Mix the well contents by tapping the plate, or shaking with a microplate shaker, for at least 5 seconds.
10. Cover with a plate sealer, then incubate at 37°C for 5 minutes.
11. Unseal the plate, then measure the OD of each well with a microplate reader at 460 nm.

C. Calculation of Results

Plot the standard curve, using the mean OD of the standard dilutions (adjusted for the blank) on the y-axis, and their respective concentrations on the x-axis. The curve should form a straight line, described by the formula $y = ax + b$.

Based on this curve, the concentration of ATP in each sample well can be derived using the following formula:

1. Tissue samples:

$$\text{ATP (µmol/kg wet weight)} = \frac{(\text{OD}_{\text{Sample}} - \text{OD}_{\text{Control}} - \text{b}) \times V}{\text{a} \times \text{M}} \times \text{F}$$

where:

$\text{OD}_{\text{Sample}}$	OD value of the sample.
$\text{OD}_{\text{Control}}$	OD value of the corresponding control
V	Volume of Extraction Solution used in the sample preparation (ml)
M	Wet weight of the tissue sample (g)
a	Gradient of the standard curve ($y = \boldsymbol{a}x + b$)
b	Y-intercept of the standard curve ($y = ax + \boldsymbol{b}$)
F	Dilution factor of the sample

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Technical Support

For troubleshooting and technical assistance, please contact us at support@abbexa.com.