Instructions for Use
Version: 1.0.1
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ATP Chemiluminescence Assay Kit

Catalog No.: abx295077

Size: 96 tests

Detection Range: 0.003 μmol/L – 5 μmol/L

Sensitivity: 0.003 μmol/L

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

Application: For detection and quantification of ATP concentration in animal tissue homogenates and cell samples.

For Reference Only

Introduction

Abbexa's ATP Chemiluminescence Assay Kit is a quick, convenient, and sensitive method for measuring and calculating ATP concentration. When ATP is catalyzed by luciferase it reacts with luciferin and produces oxyluciferin. This process emits fluorescence through bioluminescence. The intensity of the light produced is proportional to the ATP concentration, which can then be calculated.

Kit components

1. 96-well microplate
2. Extraction Solution: 2 × 50 ml
3. Enzyme Reagent: 2 vials
4. Enzyme Diluent: 14 ml
5. Standard (100 μmol/L): 1 ml
6. Plate sealer: 2

Materials required but not provided

1. Chemiluminescence Immunoassay Analyzer or a Microplate Reader which can detect chemiluminescence
2. PBS (0.01 M, pH 7.4)
3. Pipette and pipette tips
4. 1.5 ml microcentrifuge tubes
5. Centrifuge
6. Vortex mixer
7. Water bath

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Protocol

A. Preparation of samples and reagents

1. 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 amount of tissue sample needed and wash in cold PBS (0.01 M, pH 7.4). Per 50 mg of tissue, add 450 µl Extraction Solution. Homogenize manually, using a mechanical homogenizer at 4°C. Incubate in a boiling water bath for 2 minutes. Centrifuge at 10,000 × g for 10 minutes, then carefully remove the supernatant. Keep on ice for detection.
- **Cell (adherent or suspension) samples:** Harvest the number of cells needed for the assay and wash with PBS (0.01 M, pH 7.4). Per 2 × 10^6 cells, add 300 µl Extraction Solution. Homogenize with an ultrasonic cell disrupter at 4°C. Incubate in a boiling water bath for 10 minutes then immediately cool to 25°C with running water. Centrifuge at 10,000 × g for 10 minutes, then carefully remove the supernatant. Keep on ice for detection.

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 % Mouse lung tissue homogenate	1

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 kit. It is therefore recommended to use mechanical lysis methods for tissue homogenates.

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2. Reagents

Preserve Enzyme Reagent on ice during use. Equilibrate all other reagents to room temperature before use.

- **Enzyme Stock Solution:** Dissolve one vial of Enzyme Reagent with 1 ml of Enzyme Diluent and mix thoroughly. Unused enzyme Stock Solution can be stored in the dark at -20°C for up to one month.
- **Enzyme Working Solution:** Prepare enough Enzyme Working Solution needed for the amount of wells used. Add Enzyme Stock Solution to Enzyme Diluent in a ratio of 1:5 to prepare Enzyme Working Solution. For example, per 20 µl of Enzyme Stock Solution, add 100 µl of Enzyme Diluent to prepare 120 µl of Enzyme Working Solution. Prepare immediately before assaying.
- **Standards:** Label 7 tubes with 5 µmol/L, 4 µmol/L, 3 µmol/L, 2.5 µmol/L, 2 µmol/L, 1 µmol/L, and 0.5 µmol/L. Add 50 µl, 40 µl, 30 µl, 25 µl, 20 µl, 10 µl, and 5 µl of Standard (100 µmol/L) to the 5 µmol/L, 4 µmol/L, 3 µmol/L, 2.5 µmol/L, 2 µmol/L, 1 µmol/L, and 0.5 µmol/L tubes respectively, followed by 950 µl, 960 µl, 970 µl, 975 µl, 980 µl, 990 µl, and 995 µl of Extraction Solution, to prepare Standard Dilutions with concentrations 5 µmol/L, 4 µmol/L, 3 µmol/L, 2.5 µmol/L, 2 µmol/L, 1 µmol/L, and 0.5 µmol/L. These volumes are summarized in the following table:

Standard Dilution (µmol/L)	**5**	**4**	**3**	**2.5**	**2**	**1**	**0.5**	**0**
100 µmol/L **Standard (µl)**	50	40	30	25	20	10	5	0
Extraction Solution (µl)	950	960	970	975	980	990	995	1000

For the blank / 0 µmol/L standard, use pure Extraction Solution. The volume of each standard will be 1000 µl.

Note:

- Dilute samples to the optimal concentration before testing if the ATP concentration of the sample exceeds the detection range.
- Measure using no more than 30 wells (including standard and sample wells) at a time to avoid operation time differences causing inaccurate values between wells. If the number of wells to be tested is large, it is therefore recommended to measure them in batches.
- Take care to avoid bubbles when transferring supernatant to the wells.

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B. Assay Procedure

Pre-heat the incubator and ensure it has reached a stable temperature before use.

1. Assign microplate wells for each standard and sample. *It is strongly recommended to prepare all the tubes in duplicate.*
2. Add 100 µl of Enzyme Working Solution to each standard and sample well. Leave to stand for 5 minutes.
3. Add 100 µl of each Standard to the standard wells and mix thoroughly.
4. Add 100 µl of sample to the sample wells and mix thoroughly.
5. Measure the fluorescence values of each well with a chemiluminescence immunoassay analyzer or multifunctional microplate reader.

C. Calculation of Results

Average the duplicate readings for each standard. Plot the standard curve, using the fluorescence value of the standard dilutions (adjusted for the blank / 0 µmol/L standard) 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 with the following formulae:

1. Tissue samples:

$$\text{ATP concentration } (\mu\text{mol/kg wet tissue}) = f \times \frac{(F_{Sample} - F_{Blank} - b) \times V}{a \times m}$$

2. Cell samples:

$$\text{ATP concentration } (\mu\text{mol}/1\times 10^9) = f \times \frac{(F_{Sample} - F_{Blank} - b) \times V}{a \times n}$$

where:

F_{Sample}	Fluorescence value of sample
F_{Blank}	Fluorescence value of blank / 0 µmol/L standard
V	Volume of homogenate medium during the preparation of tissue or cell sample (ml)
$C_{Protein}$	Concentration of protein in sample (mg/ml)
a	Gradient of the standard curve ($y = \boldsymbol{a}x + b$)
b	Y-intercept of the standard curve ($y = ax + \boldsymbol{b}$)
m	Wet weight of sample, (0.05 g recommended)
n	Number of cells (if the number of cells is 5×10^6, n = 5)
f	The dilution factor of sample

Technical Support

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

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