Ca^{2+}-ATPase Assay Kit

Catalog No.: abx294127

Size: 100 tests

Detection Range: 0.8 U/g – 41 U/g

Sensitivity: 0.8 U/g

Storage: Store all components in the dark at 4°C.

Application: For detection and quantification of Ca^{2+}-ATPase activity in tissue homogenates and cell lysates.

Introduction

Ca^{2+}-ATPase is a transmembrane protein involved in the transportation of Ca^{2+} out of cells and therefore regulating Ca^{2+} homoeostasis within eukaryotic cells. This maintenance is required for efficient cell signaling and muscle function. Lower concentrations or activity of Ca^{2+}-ATPase can result in organs such as the heart and skeletal muscle working ineffectively.

Abbexa's Ca^{2+}-ATPase Assay Kit is a quick, convenient, and sensitive method for measuring and calculating Ca^{2+} -ATPase activity. ATPase catalyzes the production of inorganic phosphorus. The activity of Ca^{2+}-ATPase can therefore be determined by measuring the concentration of inorganic phosphorus based on the OD at 660 nm and comparing it to that of a control which has had the Ca^{2+}-ATPase activity inhibited.

Kit components

1. Buffer Solution: 20 ml
2. Substrate: 1 vial
3. Chromogenic Reagent A: 2 vials
4. Chromogenic Reagent B: 2 vials
5. Activation Reagent A: 2 × 2 ml
6. Activation Reagent B: 2 × 2 ml
7. Protein Precipitator: 10 ml
8. Acidic Reagent: 50 ml
9. Standard (10 mmol/L): 2 ml

Materials required but not provided

1. Spectrophotometer (660 nm)
2. 1 cm optical path quartz cuvettes
3. Double-distilled water
4. Normal saline (0.9% NaCl)
5. PBS (0.01 M, pH 7.4)
6. Pipette and pipette tips
7. Centrifuge and centrifuge tubes
8. Mechanical homogenizer
9. Sonicator
10. Water bath
11. Vortex mixer
12. Incubator

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 at least 50 mg of tissue, and wash with cold PBS (0.01 M, pH 7.4). Per 50 mg of tissue, add 450 µl of normal saline (0.9% NaCl) and homogenize manually, using a mechanical homogenizer at 4°C. Centrifuge at 10,000 × g for 10 minutes at 4°C and carefully collect the supernatant. Keep on ice and assay immediately.
- **Cell Lysates:** Collect at least 4 ×10^6 cells and wash with cold PBS (0.01 M, pH 7.4). Per 4 ×10^6 cells, add 500 µl of normal saline (0.9% NaCl) and homogenize using a sonicator at 4°C. Centrifuge at 10,000 × g for 10 minutes and carefully collect the supernatant. Keep on ice and assay immediately.

Note: To calculate Ca^{2+}-ATPase activity based on the protein concentration using the formulae in section **C. Calculation of Results**, the total protein concentration of the supernatant must be determined separately (**abx097193)**.

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 normal saline (0.9% NaCl), then carry out the assay procedure. The recommended dilution factor for different samples is as follows (for reference only):

Sample Type	Dilution Factor
10% Rat liver tissue homogenate	5 – 8
10% Mouse liver tissue homogenate	1
10% Rat heart tissue homogenate	5 – 8
10% Rat lung tissue homogenate	5 – 8
10% Rat kidney tissue homogenate	5 – 8
10% Rat brain tissue homogenate	2 – 3

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 cell lysates and tissue homogenates.

2. Reagents

- **Substrate Working Solution:** Dissolve 1 vial of Substrate in 10 ml of double-distilled water and mix thoroughly. Unused Substrate Working Solution can be stored at 4°C for up to 7 days.
- **Chromogenic Reagent Working Solution A:** Dissolve 1 vial of Chromogenic Reagent A in 25 ml of double-distilled water and mix thoroughly. Unused Chromogenic Reagent Working Solution A at 4°C for up to 7 days in the dark.
- **Chromogenic Reagent Working Solution B:** Dissolve 1 vial of Chromogenic Reagent B in 25 ml of double-distilled water in a 90–100°C water bath and allow to cool to room temperature. Unused Chromogenic Reagent Working Solution B can be stored at 4°C for up to 7 days.
- **Phosphorus Working Solution:** Prepare enough Phosphorus Working Solution for the tubes tested. For example, for 1 tube, prepare 2 ml of Phosphorus Working Solution. Mix thoroughly 800 µl of double-distilled water with 400 µl of Chromogenic Working Solution A, 400 µl of Chromogenic Working Solution B, and 400 µl of Acidic Reagent. Prepare for immediate use and keep in the dark.
- **0.5 µmol/L Standard:** For each well tested, prepare 200 µl of 0.5 µmol/L Standard. Mix thoroughly 10 µl of 10 mmol/L Standard with 190 µl of double-distilled water. Unused 0.5 µmol/L Standard can be stored at 4°C for up to 7 days.

Note:

- Allow all reagents to equilibrate to room temperature before use.
- Ensure any tubes used for the preparation of the reagents are free from phosphorus contamination.
- The Phosphorus Working Solution should have a pale-yellow color. If the solution appears blue or colorless it should be discarded.

B. Assay Procedure

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

1. Mark tubes for the control tube and enzyme tube.
2. Prepare each tube as per the following:

Reagent	Sample Tube	Control Tube
Buffer Solution (µl)	170	170
Activation Reagent A (µl)	0	40
Activation Reagent B (µl)	40	0
Substrate Working Solution (µl)	40	40
Sample (µl)	200	0

3. Mix thoroughly using a vortex mixer and incubate the tubes at 37°C for 10 minutes.
4. Add 50 µl of Protein Precipitator to the control tube and mix thoroughly.
5. Add 200 µl of sample to the control tube and mix thoroughly.

6. Add 50 µl of Protein Precipitator to the sample tube and mix thoroughly.
7. Centrifuge tubes at 2000 × g for 10 minutes then carefully collect the supernatant.
8. Mark tubes for each standard, sample, control and blank. Each sample requires a corresponding control. *It is strongly recommended to prepare all the wells in duplicate.*
9. Add 200 µl of double-distilled water to the blank tubes.
10. Add 200 µl of 0.5 µmol/L Standard to the standard tubes.
11. Add 200 µl of the control tube supernatant to the control tubes.
12. Add 200 µl of the sample tube supernatant to the sample tubes.
13. Add 2 ml of Phosphorus Working Solution to all tubes.
14. Mix each tube thoroughly and incubate at 37°C for 30 minutes.
15. Set the spectrophotometer to 0 using double-distilled water and measure the OD of each tube at 660 nm using 1 cm optical path quartz cuvettes.

Note:

- When conducting the preparation of the reagents and samples, and the assay procedure, take care to avoid contamination from external phosphorus.

C. Calculation of Results

1. Tissue samples:

Ca^{2+}-ATPase activity in tissue samples can be calculated according to total protein concentration (which must be assayed separately) or according to sample weight.

Total Protein

One unit of Ca^{2+}-ATPase activity is defined as the amount required for 1 mg of tissue protein to produce 1 µmol of inorganic phosphorus in 1 hour at 37°C.

$$\mathbf{Ca^{2+}\text{-}ATPase\ (U/mg\ protein)} = \frac{(\mathbf{OD_{Sample}} - \mathbf{OD_{Control}}) \times \mathbf{C_{Standard}} \times 6}{(\mathbf{OD_{Standard}} - \mathbf{OD_{Blank}})} \times \frac{\mathbf{V_{Total}}}{\mathbf{V_{Sample}} \times \mathbf{C_{Protein}}} \times \mathbf{F}$$

Sample Weight

One unit of Ca^{2+}-ATPase activity is defined as the amount required for 1 g of tissue to produce 1 µmol of inorganic phosphorus I 1 hour at 37°C.

$$\mathbf{Ca^{2+}\text{-}ATPase\ (U/g\ tissue)} = \frac{(\mathbf{OD_{Sample}} - \mathbf{OD_{Control}}) \times \mathbf{C_{Standard}} \times 6}{(\mathbf{OD_{Standard}} - \mathbf{OD_{Blank}})} \times \frac{\mathbf{V_{Total}}}{\mathbf{V_{Sample}}} \times \frac{\mathbf{V_{Saline}}}{\mathbf{W}} \times \mathbf{F}$$

2. Cell Lysates:

One unit of Ca^{2+}-ATPase activity is defined as the amount required for 10^6 of tissue to produce 1 μmol of inorganic phosphorus in 1 hour at 37°C.

$$\mathbf{Ca^{2+}\text{-}ATPase\ (U/kg\ tissue)} = \frac{(\mathbf{OD_{Sample}} - \mathbf{OD_{Control}}) \times \mathbf{C_{Standard}} \times 6}{(\mathbf{OD_{Standard}} - \mathbf{OD_{Blank}})} \times \frac{\mathbf{V_{Total}}}{\mathbf{V_{Sample}}} \times \frac{\mathbf{V_{Saline}}}{\mathbf{N}} \times \mathbf{F}$$

where:

OD_{Sample}	OD value of sample
$OD_{Control}$	OD value of control
$OD_{Standard}$	OD value of standard
OD_{Blank}	OD value of blank
$C_{Standard}$	Concentration of standard (0.5 μmol/L)
V_{Total}	Total volume in each well at final incubation (0.5 ml)
V_{Sample}	Volume of sample added to each well (0.2 ml)
V_{Saline}	Volume of normal saline (0.9% NaCl) added to tissue homogenate (ml)
$C_{Protein}$	Concentration of protein in sample (mg/ml)
W	The weight of the tissue sample (0.1 g)
N	The number of cells in the sample/10^6
F	The dilution factor of sample

Technical Support

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