Tartrate Resistant Acid Phosphatase Assay Kit

Catalog No.: abx295114

Size: 96 tests

Detection Range: 0.911 U/L – 100 U/L

Sensitivity: 0.911 U/L

Storage: Store all components at -20 °C. Store the Substrate and Standard in the dark.

Application: For detection and quantification of Tartrate Resistant Acid Phosphatase activity in serum, plasma, urine, tissue homogenates, and cell lysates.

Introduction

Abbexa's Tartrate Resistant Acid Phosphatase Assay Kit is a quick, convenient, and sensitive method for measuring and calculating Tartrate Resistant Acid Phosphatase activity. In the presence of tartaric acid, Tartrate Resistant Acid Phosphatase catalyzes the conversion of the chromogenic reagent, producing p-nitrophenol, which has an absorbance maximum at 405 nm. The intensity of the color is proportional to the Tartrate Resistant Acid Phosphatase activity, which can then be calculated.

Kit components

1. 96-well microplate
2. Buffer Solution: 24 ml
3. Substrate: 2 Vials
4. Tartaric Acid Solution: 2 × 1.4 ml
5. Standard: 2 Vials
6. Chromogenic Reagent: 15 ml
7. Plate sealer: 2

Materials required but not provided

1. Microplate reader (405 nm)
2. Double distilled water
3. Normal Saline (0.9 % NaCl)
4. PBS (0.01 M, pH 7.4)
5. Pipette and pipette tips
6. 1.5 ml microcentrifuge tubes
7. Centrifuge
8. Vortex mixer
9. 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.

- **Serum/Plasma/Urine:** Samples can be tested directly.
- **Tissue Homogenates:** Carefully weigh 20 mg of tissue and wash with cold PBS (0.01 M, pH 7.4), then add 180 µl Normal Saline (0.9% NaCl). Homogenize manually, using a mechanical homogenizer or by ultrasonication, in an ice water bath. Collect the tissue homogenate and centrifuge at 10,000 × g for 10 minutes at 4°C, then carefully remove the supernatant for analysis. The protein concentration in the supernatant should be determined separately **(abx097193)**.
- **Cell Lysates:** Collect 1 × 10^6 cells and wash with cold PBS (0.01 M, pH 7.4), then add 200 µl Normal Saline (0.9 % NaCl). Homogenize manually, using a mechanical homogenizer or by ultrasonication, in an ice water bath. Centrifuge at 10,000 × g for 10 minutes, then carefully remove the supernatant for analysis. The protein concentration in the supernatant should 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
Goat plasma	1
Human urine	1
Pig serum	1
Rat plasma	1 - 2
10% Mouse liver tissue homogenate	5 – 8
10% Mouse kidney tissue homogenate	3 – 5
10% Mouse lung tissue homogenate	2 – 5
10% Mouse brain tissue homogenate	3 – 5
1 × 10^6 HL-60 cells	1
1 × 10^6 293T cells	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 cell lysates and tissue homogenates.

2. Reagents

- **Substrate Working Solution:** Dissolve a vial of Substrate with 0.5 ml double distilled water, then store on ice in the dark. Prepare fresh before use, or store at -20°C in the dark for up to 2 days.
- **Reaction Working Solution:** Mix Buffer Solution with Substrate Working Solution at a 17:1 ratio, then store on ice in the dark. Prepare fresh and use within 6 hours of preparation.
- **10 mmol/L Standard Solution:** Dissolve a vial of Standard with 1 ml double distilled water and mix fully. Prepare fresh before use, or store at -20°C in the dark for up to 2 days.
- **1 mmol/L Standard Solution:** Mix Buffer Solution with 10mmol/L Standard Solution at a 9:1 ratio. Prepare fresh and use immediately, avoid exposure to light.

Note:

- Allow all reagents to equilibrate to room temperature before use.

B. Assay Procedure

1. **Production of Standard curve:** Label 7 tubes with 1.0 mmol/L, 0.9 mmol/L, 0.8 mmol/L, 0.6 mmol/L, 0.4 mmol/L, 0.3 mmol/L, and 0.2 mmol/L. Prepare Standard Dilutions as summarized in the following table:

Standard Dilution (mmol/L)	1.0	0.9	0.8	0.6	0.4	0.3	0.2
1 mmol/L Standard (µl)	200	180	160	120	80	60	40
Buffer Solution (µl)	0	20	40	80	120	140	160

   For the blank, or 0 mmol/L standard, use pure Buffer Solution. The volume of each standard will be 200 µl

2. Add 20 µl of standard, sample, and blank to wells as appropriate.
3. Add 120 µl of Reaction Working Solution to standard and sample wells.
4. Add 120 µl of Buffer Solution to blank well.
5. Add 20 µl of Tartaric Acid Solution to each well and mix fully.
6. Incubate at 37°C for 10 minutes.
7. Add 100 µl of Chromogenic Reagent to each well and mix fully.
8. Incubate at room temperature for 2 minutes, then measure the OD of each well with a microplate reader at 405 nm.

C. Calculation of Results

Plot the standard curve, using the 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 Tartrate Resistant Acid Phosphatase in each sample well can be derived with the following formulae:

1. Serum/Plasma/Urine samples:

One unit of Tartrate Resistant Acid Phosphatase activity is defined as the amount of TRAP in 1 L of serum, plasma or urine that produces 1 µmol of p-nitrophenol per minute at 37°C.

$$\textbf{TRAP activity (U/L)} = \frac{(\Delta \boldsymbol{A_{405}} - \boldsymbol{b})}{\boldsymbol{a}} \times \frac{\mathbf{1000}}{\boldsymbol{T}} \times \boldsymbol{f}$$

2. Tissue Homogenate and Cell Lysate samples:

One unit of Tartrate Resistant Acid Phosphatase activity is defined as the amount of TRAP in 1 g of tissue or cell protein that produces 1 µmol of p-nitrophenol per minute at 37°C.

$$\textbf{TRAP activity (U/g prot)} = \frac{(\Delta \boldsymbol{A_{405}} - \boldsymbol{b})}{\boldsymbol{a}} \times \frac{\mathbf{1000}}{\boldsymbol{T} \times \boldsymbol{C_{pr}}} \times \boldsymbol{f}$$

where:

y	$OD_{Standard} - OD_{Blank}$
x	Concentration of standard
a	Gradient of the standard curve ($y = \boldsymbol{a}x + b$)
b	Y-intercept of the standard curve ($y = ax + \boldsymbol{b}$)
ΔA_{405}	$OD_{Sample} - OD_{Control}$
T	Time of reaction (10 min)
C_{pr}	Concentration of protein in sample (g prot/L)
1000	1 mmol/L = 1000 µmol/L
f	Sample dilution factor