

User's Manual and Instructions

ATPase/GTPase Assay Kit

Catalog Number: Z5030043

Introduction

ATPases and GTPases catalyze the decomposition of ATP or GTP into ADP or GDP and free phosphate ion. These enzymes play key roles in transport, signal transduction, protein biosynthesis and cell differentiation.

BioChain' ATPase/GTPase Assay Kit offers a highly sensitive method for determining ATPase/GTPase activities in a microplate format. Its proprietary formulation features a single reagent for accurate determination of enzyme activity in 30 min at room temperature. The improved malachite green reagent forms a stable dark green color with liberated phosphate, which is measured on a plate reader (600 - 660 nm).

Features

High sensitivity: detection of as little of 2 pmoles of free phosphate.

Fast and convenient: single reagent, homogeneous "mix-and-measure" assay allows quantitation of enzyme activity within 30 minutes.

Robust and amenable to HTS: detection at 620nm greatly reduces potential interference by colored compounds. Z' factors of >0.7 are observed in 96-well and 384-well plates. Can be readily automated on HTS liquid handling systems.

Applications

Determination of ATPase and GTPase activity.

Drug Discovery: high-throughput screen for ATPase or GTPase inhibitors.

Components

Reagent: 50 mL

Assay Buffer: 10 mL

Standard: 1mL 1 mM phosphate

Storage condition

The reagents and standard are stable for one year when stored at 4°C.

Precautions

Reagent contains 0.27 M H₂SO₄. Normal precautions for laboratory reagents should be exercised while using the reagents. Please refer to Material Safety Data Sheet for detailed information.

Important: All reagents must be brought to room temperature before use. Before each assay, it is important to check that enzyme preparations and assay buffers do not contain free phosphate. This can be conveniently done by adding 200 μL of the Reagent to 40 μL sample solution. The blank OD values at 620 nm should be lower than 0.3. If the OD readings are higher than 0.3, check phosphate level. Lab detergents may contain high levels of phosphate. Make sure that lab wares are free from contaminating phosphate after thorough washes.

Protocol

ACTIVITY DETERMINATION IN 96-WELL PLATE

1. *Preparation of phosphate standards.* Prepare 500 μL Premix solution containing 50 μM phosphate by mixing 25 μL 1 mM phosphate standard with 475 μL distilled water. Number the tubes. Dilute standards as shown in the following Table. Pipette 40 μL standard in duplicate into wells of a clear-bottom 96-well plate.

No	Premix + H ₂ O	Final Vol (μL)	Phosphate Conc (μM)	pmoles Phosphate in 40 μL
1	200 μL + 0 μL	200	50	2,000
2	120 μL + 80 μL	200	30	1,200
3	60 μL + 140 μL	200	15	600
4	0 μL + 200 μL	200	0	0

2. *Perform a series dilution of enzyme in assay buffer.* Set up 40- μL reactions and a control with no enzyme in separate wells. Incubate the reaction for desired period of time (e.g. 30 min).

Reaction Well

20 μL Assay Buffer

10 μL enzyme

10 μL 4 mM ATP or GTP

Control Well

20 μL Assay Buffer

10 μL H₂O

10 μL 4 mM ATP or GTP

3. *Add 200 μL Reagent* and incubate 30 min at room temperature. Please note: use of a multi-channel pipette is recommended. The Reagent terminates the enzyme reaction and generates color with the free phosphate produced in the enzyme reaction.

4. *Read OD_{620nm}* on a plate reader.

5. *Enzyme activity.* Calculate ΔOD values by subtracting OD values in reaction and control wells. Choose an enzyme concentration that gives a ΔOD of 0.5 to 1, this will ensure that substrate hydrolysis (<10%) is within the linear kinetics of reaction. Compute the concentration of free phosphate produced [Pi] (μM) from the standard curve.

$$\text{Enzyme Activity} = [\text{Pi}] (\mu\text{M}) \times 40 \mu\text{L} \div (10 \mu\text{L} \times t) \quad (\text{U/L})$$

40 μL and 10 μL are the reaction volume and the enzyme volume in the assay. t is the reaction time (e.g. 30 min). 1 unit of activity is the amount of enzyme that catalyzes the production of 1 μmole of free phosphate per minute under the assay conditions.

INHIBITOR ASSAY IN 96-WELL PLATE

To evaluate an inhibitor or perform HTS, use the optimal enzyme concentration determined above. Incubate enzyme and inhibitor first for a certain period of time, before adding the substrate. At the end of reaction, add 200 μ L Reagent for phosphate determination.

Reaction Well

20 μ L Assay Buffer
5 μ L enzyme
5 μ L inhibitor
10 μ L 4 mM ATP or GTP

Control Well

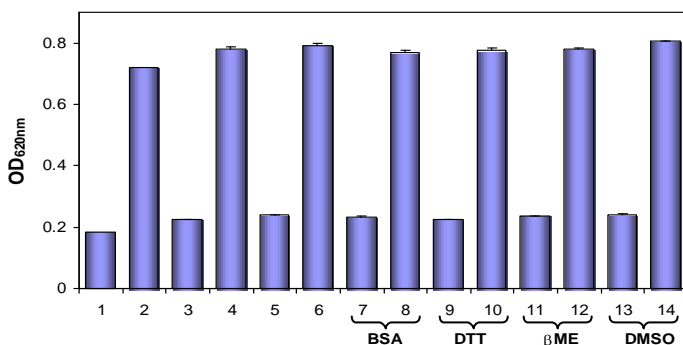
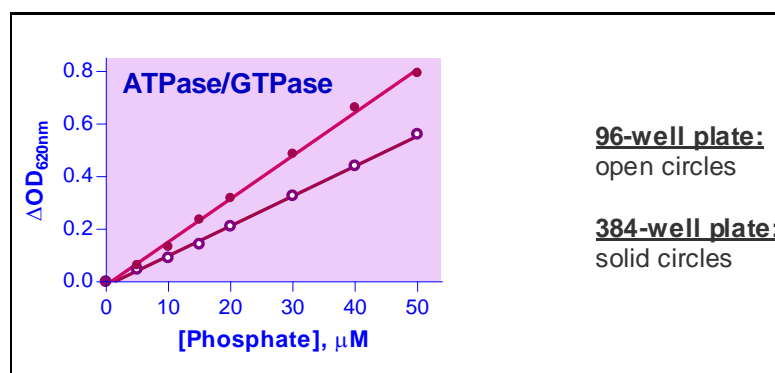
20 μ L Assay Buffer
10 μ L 4 mM ATP or GTP
10 μ L Buffer/DMSO

ASSAYS IN 384-WELL PLATE

The procedure is similar as in the 96-well plate assay, except that 20 μ L standards or 20 μ L reaction mixture (10 μ L Assay Buffer, 5 μ L 4 mM ATP, 5 μ L enzyme) are mixed with 80 μ L Reagent.

GENERAL CONSIDERATIONS

Materials. Use ultrapure ATP and GTP. The provided 2x assay buffer contains 40 mM Tris, 80 mM NaCl, 8 mM MgAc₂, 1 mM EDTA, pH 7.5. Other buffers (Hepes, Mes, Mops) can be used. Assay is compatible with 1 mM DTT, 2mM β -mercaptoethanol, 0.5 mg/mL BSA and 5% DMSO.



Phosphate assays in 96-well plate. 1. H₂O, 2. Phosphate, 3. ATP in H₂O, 4. ATP/Phosphate in H₂O, 5, 7, 9, 11, 13: ATP in Assay Buffer with, where indicated, 0.5 mg/mL BSA, 1 mM DTT, 2 mM β -mercaptoethanol (β ME) and 5% DMSO. 6, 8, 10, 12, 14: ATP/Phosphate in Assay Buffer. Phosphate and ATP were at 50 μ M and 1 mM, respectively. The assay is not affected by these components.