ATPase + GTPase Activity Assay Kit

ATPase + GTPase Activity Assay Kit can be used to measure ATPase and GTPase activity in samples.

Catalog number: ARG82139

For research use only. Not for use in diagnostic procedures.

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MANUFACTURED BY:

Arigo Biolaboratories Corporation

Address: No. 22, Ln. 227, Gongyuan Rd., Hsinchu City 300, Taiwan

Phone: +886 (3) 562 1738

Fax: +886 (3) 561 3008

Email: info@arigobio.com

INTRODUCTION

ATPases and GTPases are a class of enzymes that catalyze the decomposition of ATP or GTP into ADP or GDP and a free phosphate ion. ATPas and GTPase play important roles in transport, cell metabolism signal transduction, protein biosynthesis, cell differentiation proliferation, division and movement.
[Wikipedia: ATPases and GTPases]

PRINCIPLE OF THE ASSAY

This ATPase + GTPase Activity Assay Kit provides a simple, and rapid procedure for measuring ATPase or GTPase activity in samples. In this assay, ATPases and GTPases catalyze the decomposition of ATP or GTP into ADP or GDP and free phosphate ion. And this kit detects liberated phosphate by malachite green assay with an improved malachite green reagent and it forms a stable dark green color complex. The color intensity at 620 nm (600-660 nm) is directly proportional to the ATPase or GTPase activity in the sample. The activity of ATPase or GTPase in the sample is then determined by comparing the signals of samples to the standard.

MATERIALS PROVIDED & STORAGE INFORMATION

Component	Quantity	Storage information
Assay Buffer	10 ml (Ready to use)	-20°C
Standard (1 mM phosphate)	1 ml	-20°C
Reagent	50 ml (Ready to use)	-20°C

The kit is shipped on ice. Store all components at 4°C in dark. Shelf life of six months after receipt.

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 620 nm (600-660 nm).
- Flat bottomed 96-well microplate.
- Pipettes and pipette tips
- Deionized or distilled water.
- Use ultrapure (>99% purity) ATP and GTP.

TECHNICAL HINTS AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- The kit is shipped on ice. Store all components at 4°C in dark. Shelf life of six months after receipt.
- Assay is compatible with 1 mM DTT, 2mM beta-mercaptoethanol, 0.5 mg/mL BSA and 5% DMSO.
- Before using the kit, spin tubes and bring down all components to the bottom of tubes.
- Before each assay, it is important to check that enzyme preparations and assay buffers do not contain free phosphate.
- All materials should be equilibrated to room temperature (RT) before use.
- Ensure complete reconstitution and dilution of reagents prior to use.
- It is recommended that the standards and samples be assayed in at least duplicates.
- Change pipette tips between the addition of different reagent or samples.

REAGENT PREPARATION

- **Standard:** Adding 25 µL of 1 mM phosphate standard stock into 475 µL of distilled water to yield 500 µL of 50 µM phosphate standard solution, mix well. Dilute the 50 µM phosphate standard solution with distilled water to yield standard concentration as 50 µM, 30 µM and 15 µM.

Standard No.	Standard Conc. µM	Distilled water (µl)	Standard (µl)
S1	50	475	25 µl of 1 mM Stock
S2	30	80	120 µl of S1
S3	15	140	60 µl of S1
S0	0	100	0

- **Sample**: It is suggested to make a series dilution of sample in assay buffer. If the initial assay found samples contain ATPase/GTPase activity higher than the highest standard, the samples can be further diluted with assay buffer and then re-assay the samples. For the calculation of the concentrations this dilution factor has to be taken into account. The sample must be well mixed with the assay buffer before assay.
  (It is recommended to do pre-test to determine the suitable dilution factor).

- Free phosphate checking - it is important to check that sample preparations and assay buffers do not contain free phosphate before assay. This can be checked by adding 200 µL of the Reagent to 40 µL assay buffer diluted sample solution. The OD values of the test 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.

ASSAY PROCEDURE

All materials should be equilibrated to room temperature (RT) before use, each vial should be mixed thoroughly without foaming and briefly centrifuge tubes prior to use. Each sample requires a sample blank.

1. Add 30 µl of Assay Buffer and 10 µl of 4 mM ATP or GTP into the well in flat bottomed 96 well plate as Control.
2. Add 20 µl of Assay Buffer, 10 µl of 4 mM ATP or GTP and 10 µl of each sample in the well in flat bottomed 96 well plate.
3. Add 40 µl of Standard (S0-S3) in duplicate into the wells in flat bottomed 96 well plate.
4. Gently tap the plate to ensure thorough mixing. Incubate for 30 min at room temperature.
5. Add 200 µl of the Reagent to each well. (Note: using a multi-channel pipettor is recommended.)
6. Gently tap the plate to ensure thorough mixing. Incubate for 30 min at room temperature. (The Reagent terminates the enzyme reaction and generates color with the free phosphate produced in the enzyme reaction.)
7. Read the OD with a microplate reader at 620 nm (600 - 660nm) immediately.

Summary:

	Assayed sample well	Control well	Standard
Assay buffer	20 µl	30 µl	-
4 mM ATP or GTP	10 µl	10 µl	-
Sample	10 µl	-	-
Standard (S0-S3)	-	-	40 µl
Mix well. Incubate for 30 min at room temperature.			
Reagent	200 µl	200 µl	200 µl
Mix well and incubate for 30 min at RT.			
Read the OD with a microplate reader at 620nm immediately.			

CALCULATION OF RESULTS

1. The Control 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.
2. 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.
3. Calculate the sample ATPasr/GTPase activity as follows:

   [Enzyme Activity] (U/L)

   = [(Standard Pi concentration µM) X 40 µl] / (10 µl X t)

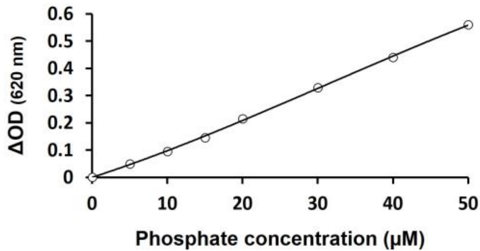
   Note:

   a. 40 µL (standard) and 10 µL (sample) are the reaction volume and the enzyme volume in the assay.

   b. t is the sample reaction time (e.g. 30 min).

4. 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.

EXAMPLE OF ASSAY



QUALITY ASSURANCE

Sensitivity

The minimum detectable dose (MDD) of ATPase/GTPase Activity was:

0.007 U/L