Technical Bulletin

ATPase/GTPase Activity Assay Kit

Catalog Number MAK113

Product Description

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

The ATPase/GTPase Activity Assay kit provides a simple and direct procedure for measuring ATPase/GTPase activity in a microplate format. This kit uses a single reagent formulation to accurately determine enzyme activity in 30 minutes at room temperature. The malachite green reagent forms a stable dark green color with free phosphate liberated by the enzymes resulting in a colorimetric product, measured at 620 nm (600–660 nm) and proportional to the enzyme activity present. This high-sensitivity kit can detect ATPase and GTPase activity levels as low as 0.007 U/L. One unit is the amount of enzyme that catalyzes the production of 1 µmole of free phosphate per minute under the assay conditions.

Components

The kit is sufficient for 200 colorimetric assays in 96-well plates.

- Reagent 50 mL
  Catalog Number MAK113A
- Phosphate Standard, 1 mM 1 mL
  Catalog Number MAK113B
- Assay Buffer 10 mL
  Catalog Number MAK113C

Reagents and Equipment Required but Not Provided

- Pipetting devices and accessories (e.g., multichannel pipettor)
- Spectrophotometric multiwell plate reader
- Clear flat-bottom 96-well plates. Cell culture or tissue culture treated plates are **not** recommended.
- 1.5 mL microcentrifuge tubes
- ATP, Adenosine 5′-triphosphate (Catalog Number A7699 or equivalent) and/or GTP, Guanosine 5′-triphosphate. Note: It is recommended to use high purity ATP/GTP product (≥99%) as the kit is highly sensitive to impurities.

Precautions and Disclaimer

For R&D use only. Not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

The kit is shipped at room temperature. Store components at 2-8 °C, protected from light.

Preparation Instructions

Briefly centrifuge vials before opening. Bring all reagents to room temperature prior to assay. Use purified water for the preparation of reagents and standards.

Before each assay, it is important to check that enzyme preparations and assay buffers do not contain free phosphate. To test for free phosphate, add 200 µL of the Reagent to 40 µL sample solution. The blank absorbance values at 620 nm should be lower than 0.3. If the absorbance readings are higher, free phosphate may be a problem and phosphate levels should be checked. Lab detergents may be one source of phosphate contamination. Ensure that labware is free from contaminating phosphate by rinsing thoroughly.

The kit is **not** recommended for use with crude sources of ATPase/GTPase, e.g., cell lysates or tissue, due to the possible presence of free phosphate.

Note: The provided Assay Buffer contains 40 mM Tris, 80 mM NaCl, 8 mM $MgAc_2$, 1 mM EDTA, pH 7.5. Other buffers (HEPES, MES, MOPS), with the exception of phosphate buffers, can be used. Assay is compatible with 2 mM β-mercaptoethanol, 1 mM DTT, 0.5 mg/mL BSA, and 5% DMSO.

Prepare a 4 mM solution of ATP or GTP in purified water. Aliquot solution and store at -20 °C. Solutions should be used within 2 months of preparation.

Procedure

All samples and standards should be run in duplicate.

Standard Curve Preparation

Note: A new standard curve must be set up each time the assay is run.

1. Prepare a 50 µM Phosphate Standard by mixing 25 µL of the 1 mM Phosphate Standard with 475 µL of purified water.
2. Prepare Phosphate standards in 1.5 mL microcentrifuge tubes according to Table 1.

Table 1.
Preparation of Phosphate Standards

Well	50 µM Phosphate Standard	Purified Water	Phosphate (µM)	pmoles Phosphate in 40 µL
1	200 µL	-	50	2,000
2	120 µL	80 µL	30	1,200
3	60 µL	140 µL	15	600
4	-	200 µL	0	0

3. Mix well and transfer 40 µL of each Standard into separate wells of a 96-well plate.

Sample Preparation

For unknown enzyme samples, it is suggested to test several sample volumes to make sure the readings are within the standard curve range.

Add 1–10 µL of Sample enzyme (ATPase and/ or GTPase) into duplicate wells of the plate. Adjust the volume of each well to 10 µL with Assay Buffer.

No Enzyme Control

Add 10 µL of Assay Buffer into duplicate wells of the plate.

Assay Reaction

1. Mix enough reagents for the number of assays to be performed. For each Sample and No Enzyme Control well, prepare 30 µL of the appropriate Reaction Mix according to Table 2.

Table 2.
Preparation of Reaction Mix

Reagent	Sample Wells	No Enzyme Control Wells
Assay Buffer	20 µL	30 µL
4 mM ATP or GTP	10 µL	-

2. Add 30 µL of appropriate Reaction Mix to each Sample and No Enzyme Control well. Do **not** add to the Standard wells.
3. Incubate the reaction for desired period of time (e.g., 30 minutes) at room temperature. Optimal time may need to be determined experimentally.
4. Add 200 µL of Reagent to **each** well and incubate for 30 minutes at room temperature to terminate the enzyme reaction and generate the colorimetric product. For best results, it is recommended to use a multichannel pipettor.

Measurement

Read the optical density (OD) of all wells at 620 nm.

Results

1. Create a standard curve by plotting the OD values of the Standards against their respective concentrations.
2. Calculate ΔOD values by subtracting the No Enzyme Control OD value from each Sample OD value.
3. Choose an enzyme concentration that gives a ΔOD of 0.5 to 1.0, this will ensure that substrate hydrolysis (<10%) is within the linear kinetics of the reaction.
4. Determine the concentration of free phosphate produced [Pi] (µM) from the standard curve.
5. Calculate the ATPase/GTPase Activity:

Enzyme Activity (U/L) =

$$\frac{Pi\ (\mu M)\ x\ 40\ \mu L}{S_V\ x\ T}$$

where

40 µL = Reaction Volume

S_V = Sample volume (µL) added to well

T = Reaction time (minutes)

Unit Definition: One unit is the amount of enzyme (ATPase or GTPase) that catalyzes the production of 1 µmole of free phosphate per minute under the assay conditions.

Enzyme Inhibitor Assay Reaction

1. To evaluate an inhibitor or perform high-throughput screening (HTS), use the optimal enzyme concentration determined above and set up reactions according to the scheme in Table 3. Incubate the enzyme and inhibitor together. The amount of time required for the inhibitor to inactivate the enzyme may need to be experimentally determined.

Table 3.
Inhibitor Assay Reaction Mixes

Reagent	Samples	Control Well
Assay Buffer	20 µL	20 µL
Enzyme Sample	5 µL	-
Inhibitor	5 µL	-
Buffer/DMSO	-	10 µL

2. Following this incubation, add 10 µL of the substrate (4 mM ATP or GTP) to all wells and incubate the reaction for the amount of time used in Step 3 of the Enzyme Activity Assay Reaction.

3. Continue the assay from Step 4 of the Enzyme Activity Assay Reaction.

Enzyme and Inhibitor Assay In 384-Well Plates

The procedure is similar as in the 96-well plate assay, except that 20 µL Standards or 20 µL Reaction Mixture (10 µL Assay Buffer, 5 µL 4 mM ATP, 5 µL enzyme) are mixed with 80 µL Reagent.

Figure 1.
Typical Phosphate Standard Curve

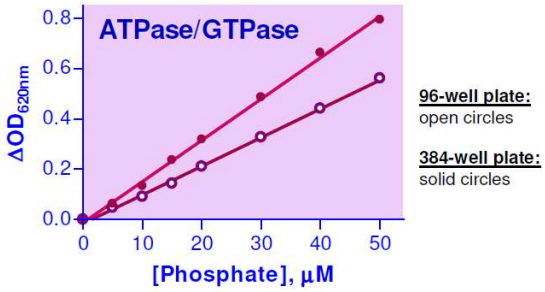
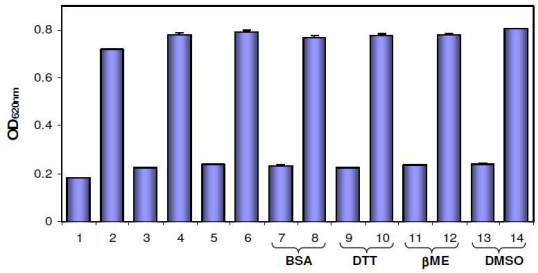


Figure 2.
Effects of possible interfering substances. 1. H_2O, 2. Phosphate, 3. ATP in H_2O, 4. ATP/Phosphate in H_2O, 5, 7, 9, 11, 13: ATP in Assay Buffer with, where indicated, 0.5 mg/mL BSA, 1 mM DTT, 2 mM β-mercaptoethanol (bME) and 5% DMSO. 6, 8, 10, 12, 14: ATP/Phosphate in Assay Buffer. Phosphate and ATP were at 50 µM and 1 mM, respectively. The assay is not affected by these components.



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