Technical Manual

ChromaDazzle ATPase/GTPase Activity Assay Kit

Catalogue Code: BA0017

Pack Size: 200 assays

Research Use Only

DESCRIPTION

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.

The Assay Genie ChromaDazzle ATPase/GTPase Activity 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).

KEY FEATURES

High sensitivity: detection of 0.007 U/L ATPase or GTPase activity.
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.

KIT CONTENTS: 200 ASSAYS IN 96-WELL PLATE

Reagent: 50 mL **Assay Buffer**: 10 mL
Standard: 1mL 1 mM phosphate

Storage conditions. The kit is shipped at room temperature. The reagents and standard are stable for one year when stored at 4°C.

Precautions: reagent contains 0.27 M H_2SO_4. 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.*

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_2O	Final Vol (µL)	Phosphate Conc (µM)	pmoles Phosphate in 40 µL
1	200µL + 0µL	200	50	2,000
2	120µL + 80µL	200	30	1,200
3	60µL + 140µL	200	15	600
4	0µL + 200µL	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	Control Well
20 µL Assay Buffer	20 µL Assay Buffer
10 µL enzyme	10 µL H_2O
10 µL 4 mM ATP or GTP	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 pipettor is recommended. The Reagent terminates the enzyme reaction and generates color with the free phosphate produced in the enzyme reaction.

4. *Read OD620nm* 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.

Enzyme Activity = [Pi] (µM) x 40 µL ÷ (10 µL x *t*) (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 µL Reagent for phosphate determination.

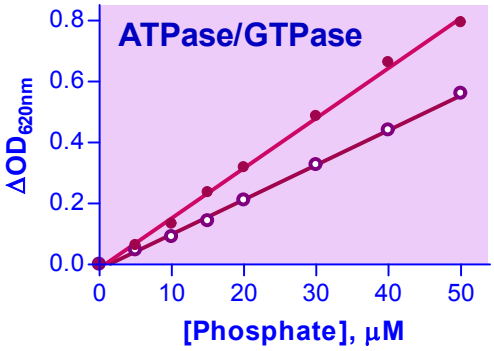
Reaction Well	Control Well
20 µL Assay Buffer	20 µL Assay Buffer
5 µL enzyme	10 µL 4 mM ATP or GTP
5 µL inhibitor	10 µL Buffer/DMSO
10 µL 4 mM ATP or GTP	

ASSAYS IN 384-WELL PLATE

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.

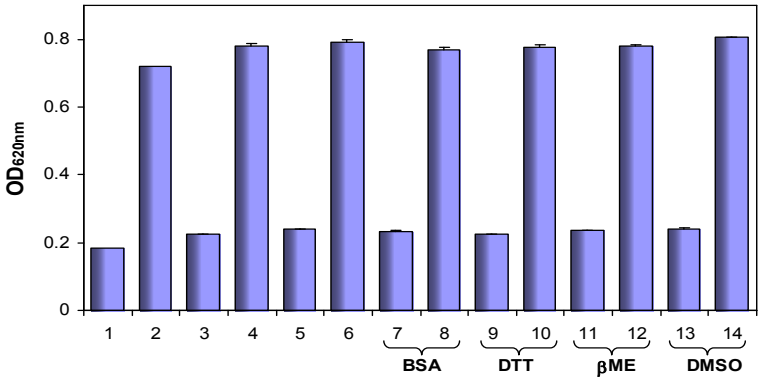
GENERAL CONSIDERATIONS

Materials. Use ultrapure ATP and GTP. The provided 2x assay buffer contains 40 mM Tris, 80 mM NaCl, 8 mM $MgAc_2$, 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.



96-well plate: open circles

384-well plate: solid circles



Phosphate assays in 96-well plate. 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 (βME) 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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