QS S Assist KINASE_ADP-Glo™ Kit

Description

KINASE ADP-Glo™ kit is designed for use in biochemical kinase assays based on a Luminescent ADP Detection Assay (ADP-Glo™). The kit includes human kinase, substrate solution, $MgCl_2$, assay buffer, kinase dilution buffer, and a protocol to perform 384 well plate assays. To detect the reaction, a luminescence microplate reader suitable for 384 well plates is required.

Components (400 dpt x 1 set)

Materials	Volume	Storage
10 x Assay Buffer	2.5 mL x 1	-80°C
10 x Kinase Dilution Buffer	1.4 mL x 1	-80°C
KINASE *	13 µL x 1	-80°C
Substrate Solution	2.6 mL x 1	-80°C
1 M $MgCl_2$	250 µL x 1	-80°C

* Concentration of the kinase; 200 µg/mL

Please avoid repeated freeze-thaw cycles.

Reagent Preparation (per 400 dpt)

Bring all reagents (except kinases) to room temperature before use.

Materials provided

Assay Buffer

Thaw 10 x Assay Buffer and take 2 mL. Dilute with 18 mL of distilled water. Diluted Assay Buffer can be kept at room temperature before use. Please do not use thawed assay buffer for more than one day, because the buffer component DTT is unstable.

Kinase Dilution Buffer

Thaw 10 x Kinase Dilution Buffer and take 1 mL. Dilute with 9 mL of distilled water. Diluted Kinase Dilution Buffer can be kept at room temperature before use. Please do not use thawed kinase dilution buffer for more than one day, because the buffer component DTT is unstable.

Substrate Solution

Thaw Substrate Solution and mix thoroughly by vortexing or sonication (5min) until dissolved.

Remaining Substrate Solution should be aliquoted and stored at -80°C.

Enzyme Solution

Thaw KINASE and dilute it appropriate-fold with Kinase Dilution Buffer. Keep on ice before use.

Materials required

384 Well Low Flange Black Flat Bottom Polystyrene（Corning #3573）

Typically, white plates are recommended for use with the ADP-Glo™ assay by Promega. However, to avoid crosstalk from vicinity wells, we recommend using black plates.

Polystyrene Universal Microplate Lid without Corner Notch, Sterile (Corning #3098)

Plate shaker

Sufficient mixing is necessary to obtain reproducible data.

Compound Solution

Prepare a hundred times concentrated (100x) compound stock solution with DMSO. Dilute the solution 25 times with Assay Buffer. For the vehicle control, prepare 4% DMSO-Assay Buffer solution.

ADP-Glo™ Kinase Assay (Promega, No. V9102)

ATP Solution

Thaw Ultra Pure ATP, 10 mM, provided from Promega's "ADP-Glo™ Kinase Assay" and dilute it to appropriate concentration with Assay Buffer.

ADP-Glo™ Reagent/Mg

Thaw ADP-Glo™ Reagent provided from Promega's "ADP-Glo™ Kinase Assay" and 1 M $MgCl_2$. For each plate (384 wells), add 100 µL of 1 M $MgCl_2$ to 9.9 mL of ADP-Glo™ Reagent (final 10 mM $MgCl_2$).

Kinase Detection Reagent (Follow Promega's "ADP-Glo™ Kinase Assay" instructions.)

Equilibrate the Kinase Detection Buffer and Kinase Detection Substrate to room temperature before use. Transfer the entire volume of Kinase Detection Buffer into the amber bottle containing Kinase Detection Substrate to reconstitute the lyophilized substrate. This forms the Kinase Detection Reagent. Mix by gently vortexing, swirling or inverting the contents to obtain a homogeneous solution. The Kinase Detection Substrate should go into solution in less than one minute. The Kinase Detection

Reagent should be used immediately or dispensed into aliquots and stored at –20°C.

Generating a Standard Curve for Conversion of ATP to ADP (Follow Promega's "ADP-Glo™ Kinase Assay" instructions)

To estimate the amount of ADP produced in kinase reactions, it is recommended to first create a standard curve that represents the luminescence corresponding to the conversion of ATP to ADP (the "ATP-to-ADP conversion curve") based on the ATP concentration used in the kinase reaction. These conversion curves represent the amounts of ATP and ADP present in a reaction at the specified conversion percentage.

The standard samples used to generate an ATP-to-ADP conversion curve are created by combining the appropriate volumes of ATP and ADP stock solutions.

<Procedure>

(1) Prepare 1 ml of 100 µM ATP and 500 µl of 100 µM ADP by diluting the Ultra Pure ATP and ADP supplied from Promega's "ADP-Glo™ Kinase Assay" in a suitable Assay Buffer. Use only Promega's Ultra Pure ATP when performing the ADP-Glo™ Kinase Assay. Other sources of ATP may contain ADP that could result in high background.

(2) Combine the 100 µM ATP and 100 µM ADP solutions prepared in Step 1 to prepare the following ATP and ADP mixtures:

100 µM ADP	100	80	60	40	20	10	5	4	3	2	1	0	(µL)
100 µM ATP	0	20	40	60	80	90	95	96	97	98	99	100	(µL)
%ADP	100	80	60	40	20	10	5	4	3	2	1	0	

(3) Transfer 20 µL of each sample into separate wells of an assay plate.

(4) Follow the below "ASSAY PROCEDURE", starting at Step 3.

Example of Reaction Mixture

Sample	Compound Solution (µL)	Vehicle (µL)	ATP Solution (µL)	Substrate Solution (µL)	Enzyme Solution (µL)	Kinase Dilution Buffer (µL)
A	–	5	5	5	–	5
B	–	5	5	5	5	–
C	5	–	5	5	5	–

Where A equals negative control, B equals positive control and C equals test sample.

Calculate the percent inhibition of compound as follows;

Inhibition (%)＝(1－(C－A)/(B－A)) x 100

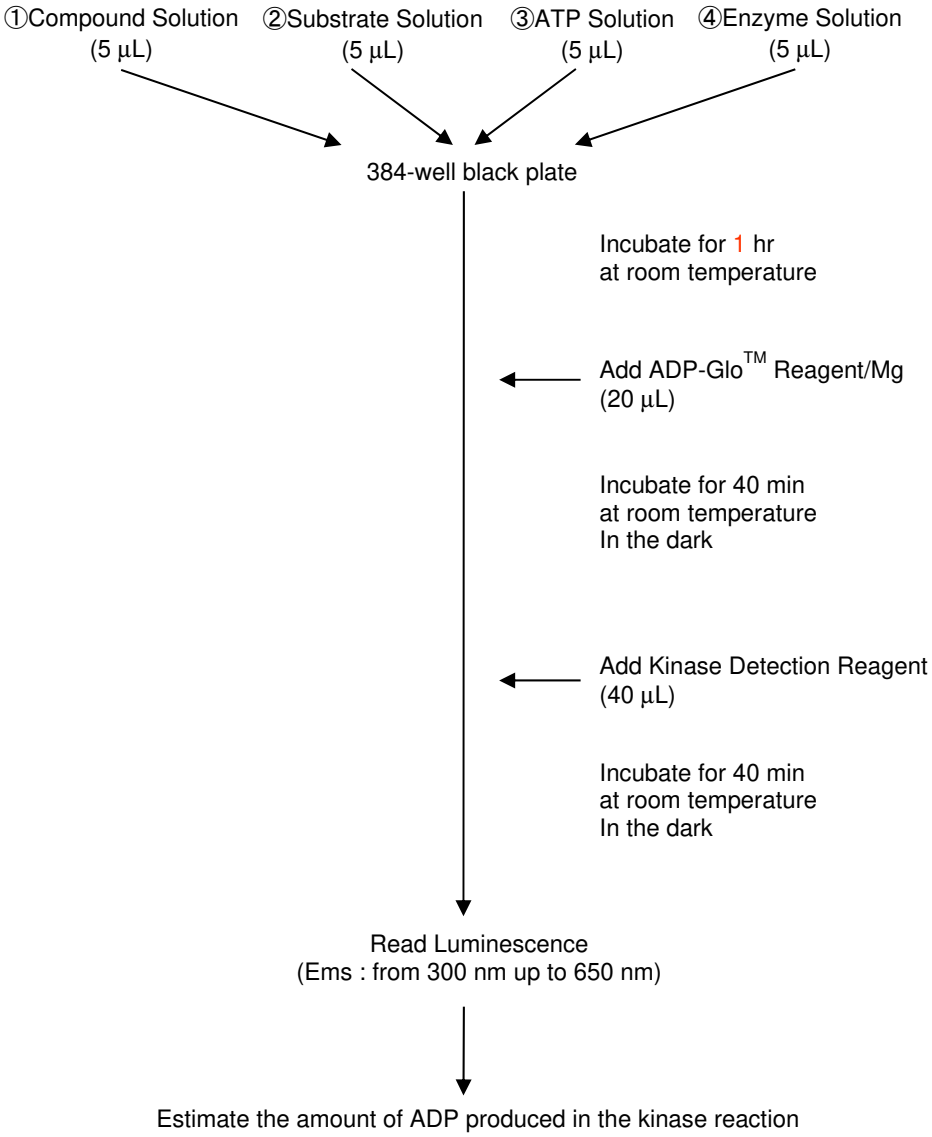
Assay Buffer composition : 50 mM MOPS, 1 mM DTT, pH7.2

Other main constitution : 250 nM Substrate, 50 μM ATP, 10 mM Metal

Detection Mode (2104 EnVision, Revvity)

Parameter	Setting
Mirror	Luminescence
Emission filter	Luminescence 700
Measurement height	6 mm
Measurement time	0.25 s

Illustration of Assay Procedures:



ASSAY PROCEDURE:

All procedures are performed at room temperature.

1. Add 5 µL of Compound Solution, 5 µL of Substrate Solution, 5 µL of ATP Solution to the assay plate, and then add 5 µL of Enzyme Solution to start kinase reaction. Incubate for 1 hour at room temperature in the dark.

2. Prepare the ATP/ADP Mixtures for generating an ATP-to-ADP conversion curve. Transfer 20 µL of each %ADP sample into the assay plate.

3. Add 20 µL of ADP-Glo™ Reagent/Mg to each well. Incubate for at least 40 minutes (the signal is stable up to 120 minutes) at room temperature with light shielding.

4. Add 40 µL of Kinase Detection Reagent to each well. Incubate for at least 40 minutes (the signal is stable up to 120 minutes) at room temperature with light shielding.

5. Measure the luminescence with a plate-reading luminometer.

6. Create an ATP-to-ADP conversion curve from the luminescence of each ATP/ADP Mix sample (Step 2).

Assay result example

The inhibitory effect of Reference compound on KINASE evaluated with KINASE ADP-Glo™ kit is shown below.