

# ADP-Glo™ Kinase Assay

INSTRUCTIONS FOR USE OF PRODUCTS V9101, V9102 AND V9103.

Quick  
PROTOCOL

For more information about the ADP-Glo™ Kinase Assay, see the *ADP-Glo™ Kinase Assay Technical Manual #TM313* available at: [www.promega.com/tbs/](http://www.promega.com/tbs/)

## Preparing the Kinase Detection Reagent

### Kinase Detection Buffer Preparation

1. Thaw Kinase Detection Buffer at room temperature, and observe for the presence of precipitate.

**Note:** There is no observed change in performance of the ADP-Glo™ Kinase Assay if the buffer contains a precipitate. However, to avoid clogging the pipette tips, the precipitate may be removed or solubilized according to Step 2.

2. If a precipitate is present, incubate the Kinase Detection Buffer at 37°C with constant swirling for 15 minutes to dissolve the precipitate. Alternatively, remove the precipitate from the Kinase Detection Buffer by carefully pipetting the supernatant from the bottle.

### Kinase Detection Reagent Preparation

1. Equilibrate the Kinase Detection Buffer and Kinase Detection Substrate to **room temperature** before use.
2. 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.
3. 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.
4. The Kinase Detection Reagent should be used immediately or dispensed into aliquots and stored at -20°C. See the *ADP-Glo™ Kinase Assay Technical Manual #TM313* for reagent stability information.

## Generating a Standard Curve for Conversion of ATP to ADP

To estimate the amount of ADP produced in the kinase reaction, we recommend creating 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 standard curves represent the amounts of ATP and ADP available in a reaction at the specified conversion percentage. The standard samples used to generate an ATP-to-ADP standard curve are created by combining the appropriate volumes of ATP and ADP stock solutions (Table 1).

1. Prepare 1ml of 1mM ATP and 500µl of 1mM ADP by diluting the supplied Ultra Pure ATP and ADP in preferred 1X kinase reaction buffer. (See Technical Manual #TM313 for a recommended kinase buffer recipe.)
2. Add 90µl of 1X kinase reaction buffer to wells B1–B12, C1–C12 and D1–D12 of a 96-well plate.
3. Combine the 1mM ATP and 1mM ADP solutions prepared in Step 1 in wells A1–A12 as indicated in Table 1 to simulate the ATP and ADP concentrations at each percent ADP (see Technical Manual #TM313). Mix well. This is the 1mM series.

**Table 1. Preparation of the 1mM Series of ATP+ADP Standards.**

Well Number	A1	A2	A3	A4	A5	A6	A7	A8	A9	A10	A11	A12
1mM ADP (µl)	100	80	60	40	20	10	5	4	3	2	1	0
1mM ATP (µl)	0	20	40	60	80	90	95	96	97	98	99	100

4. See the *ADP-Glo™ Kinase Technical Manual #TM313* for instructions on additional dilutions for the 100µM, 10µM and 1µM series of ATP+ADP Standards, available online at: [www.promega.com/tbs/](http://www.promega.com/tbs/)

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## Performing the ADP-Glo™ Kinase Assay

The ADP-Glo™ Kinase Assay is performed in two steps once the kinase reaction is complete. For 384-well plates, we recommend using 5µl of kinase reaction, 5µl of ADP-Glo™ Reagent and 10µl of Kinase Detection Reagent for a total volume of 20µl. Other volumes may be used, provided the 1:1:2 ratio of kinase reaction volume to ADP-Glo™ Reagent volume to Kinase Detection Reagent volume is maintained. The ADP-Glo™ Kinase Assay Protocol for 384-well plates is outlined below.

### ADP-Glo™ Kinase Assay Protocol

1. Perform a 5µl kinase reaction using 1X kinase buffer.  
**Note:** If the kinase reaction was not run at room temperature, equilibrate the plate to room temperature before adding the ADP-Glo™ Reagent.
2. Add 5µl of ADP-Glo™ Reagent to stop the kinase reaction and deplete the unconsumed ATP, leaving only ADP and a very low background of ATP.  
**Note:** The final Mg<sup>2+</sup> concentration should not be less than 0.5mM.
3. Incubate at room temperature for 40 minutes.
4. Add 10µl of Kinase Detection Reagent to convert ADP to ATP and introduce luciferase and luciferin to detect ATP.
5. Incubate at room temperature for 30–60 minutes, depending on the ATP concentration used in the kinase reaction (see Table 2).

**Table 2. Incubation Time to Convert ADP to ATP.**

ATP Concentration	10–100µM	100–500µM	500–1,000µM
Time	30 minutes	40 minutes	60 minutes

6. Measure the luminescence with a plate-reading luminometer or charge-coupled device (CCD) camera.  
**Note:** Instrument settings depend on the manufacturer. An integration time of 0.25–1 second per well should serve as a guideline. The long half-life of the ADP-Glo™ Kinase Assay signal allows plates to be left longer at room temperature before reading if desired.

### Additional Protocols

To screen for inhibitors or to determine IC<sub>50</sub> values of kinase inhibitors using the ADP-Glo™ Kinase Assay, see the *ADP-Glo™ Kinase Assay Technical Manual #TM313* for protocol information.

For complete protocol information, see the *ADP-Glo™ Kinase Assay Technical Manual*, available online at: [www.promega.com/tbs/](http://www.promega.com/tbs/)

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