



Promega

Technical Manual

ADP-Glo™ Kinase Assay

INSTRUCTIONS FOR USE OF PRODUCTS V9101, V9102 AND V9103.

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ADP-Glo™ Kinase Assay

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 of this system. E-mail: techserv@promega.com

1. Description	1
2. Product Components and Storage Conditions	9
3. Preparing for the ADP-Glo™ Kinase Assay	10
A. Preparing the Kinase Detection Reagent	10
B. Generating a Standard Curve for Conversion of ATP to ADP	11
4. ADP-Glo™ Kinase Assay Protocols	12
A. Kinase Reaction Buffer Considerations.....	13
B. ADP-Glo™ Kinase Assay Protocol.....	13
C. Optimizing Kinase Reaction Conditions	14
D. Screening for Inhibitors Using the ADP-Glo™ Kinase Assay.....	15
E. Determining IC ₅₀ Values of Kinase Inhibitors	16
5. General Considerations	17
6. References	18
7. Composition of Buffer	19
8. Related Products	19

1. Description

The ADP-Glo™ Kinase Assay^(a-e) is a luminescent ADP detection assay that provides a universal, homogeneous, high-throughput screening method to measure kinase activity by quantifying the amount of ADP produced during a kinase reaction. The ADP-Glo™ Kinase Assay can be used to monitor the activity of virtually any ADP-generating enzyme (e.g., kinase or ATPase) using up to 1mM ATP. The ADP-Glo™ Kinase Assay is performed in a multiwell plate and can detect kinase activity in a reaction volume as low as 5µl. The assay is performed in two steps; first, after the kinase reaction, an equal volume of ADP-Glo™ Reagent is added to terminate the kinase reaction and deplete the remaining ATP. Second, the Kinase Detection Reagent is added to simultaneously convert ADP to ATP and allow the newly synthesized ATP to be measured using a luciferase/luciferin reaction (Figures 1 and 2). The light generated is measured using a luminometer. Luminescence can be correlated to ADP concentrations by using an ATP-to-ADP conversion curve (Figure 3). This assay is sensitive enough to detect very low amounts of ADP (20nM) and can detect generated ADP in a reaction containing up to 1mM ATP in a linear



fashion (Figure 3). The luminescent signal generated is proportional to the ADP concentration produced and is correlated with kinase activity (Figure 4). Furthermore, Figure 4, Panel B, shows the high assay performance for kinases with low ATP turnover. The ADP-Glo™ Kinase Assay can be performed with virtually any kinase and substrate combination and does not require radioactively labeled components or antibodies. The kinase substrate can be a peptide, protein, lipid or sugar. Because of its high sensitivity, the ADP-Glo™ Kinase Assay can be used with essentially all kinases and especially kinases with low enzyme turnover, like growth factor receptor tyrosine kinases. Therefore, the assay is ideal for high-throughput screening because a small amount of enzyme and low ATP-to-ADP conversion generate a high signal-to-background ratio. (We define SB₅, SB₁₀ and SB₂₀ as the amounts of enzyme needed to achieve signal-to-background ratios of 5, 10 and 20, respectively.)

The ADP-Glo™ Kinase Assay relies on the properties of a proprietary thermostable luciferase (Ultra-Glo™ Recombinant Luciferase) that is formulated to generate a stable “glow-type” luminescent signal and improve performance across a wide range of assay conditions. The signal, which is produced by the luciferase reaction initiated by adding the Kinase Detection Reagent, is stable for more than 3 hours (data not shown). This extended stability eliminates the need for a luminometer equipped with injectors and allows batch-mode processing of multiple plates. In addition, the unique combination of Ultra-Glo™ Recombinant Luciferase and proprietary formulations of the ADP-Glo™ Reagent and Kinase Detection Buffer result in luminescence that is much less susceptible to interference from library compounds than other luciferase- or fluorescence-based assays (1,2). In addition to providing biochemical values (e.g., K_m of ATP or K_m of substrate) comparable to those reported in the literature, the ADP-Glo™ Kinase Assay can be used to distinguish between ATP competitive and noncompetitive kinase inhibitors (Figure 5).

Advantages of the ADP-Glo™ Kinase Assay

- **Broad linear range of ATP concentrations (micromolar to millimolar range):** Distinguish between ATP competitive and noncompetitive inhibitors.
- **Large dynamic range:** High signal-to-background ratios at lower percent conversions of ATP to ADP allow use of smaller amounts of enzyme.
- **High sensitivity:** Detect 0.2pmol ADP with more than twofold difference over background.
- **Reliable, reproducible data:** Routinely obtain Z' factor values >0.7 even with low ATP consumption rates.
- **Universal assay:** Use any kinase and kinase:substrate combination, including peptide, protein, lipid and sugar substrates.
- **Positive correlation:** Assay signal increases linearly with increasing product formation.
- **Luminescence-based ADP detection:** Experience less overall assay interference from chemical compounds.
- **Batch plate processing:** The luminescent signal is highly stable with <20% proportional increase in signal after 3 hours.
- **Homogeneous, robust and non-radioactive assay.**

1. Description (continued)

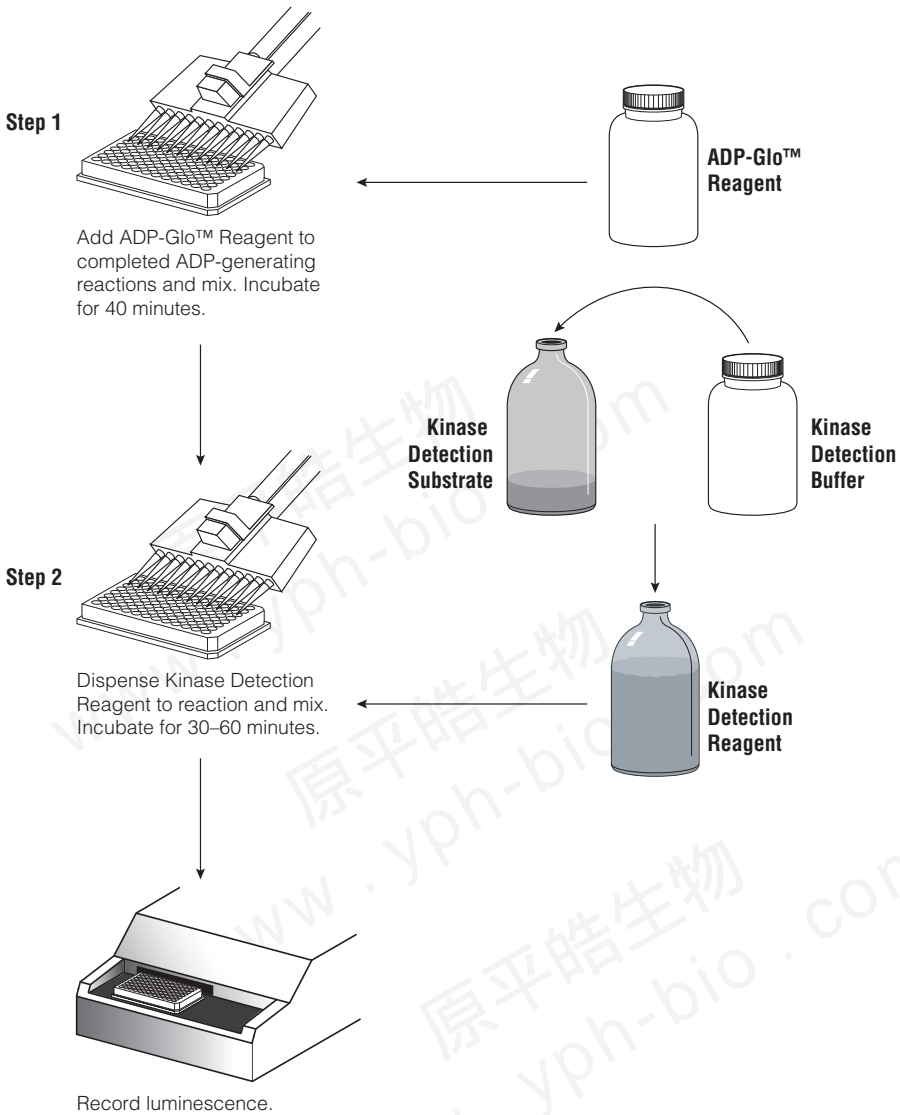


Figure 1. Schematic representation of the ADP-Glo™ Kinase Assay protocol.

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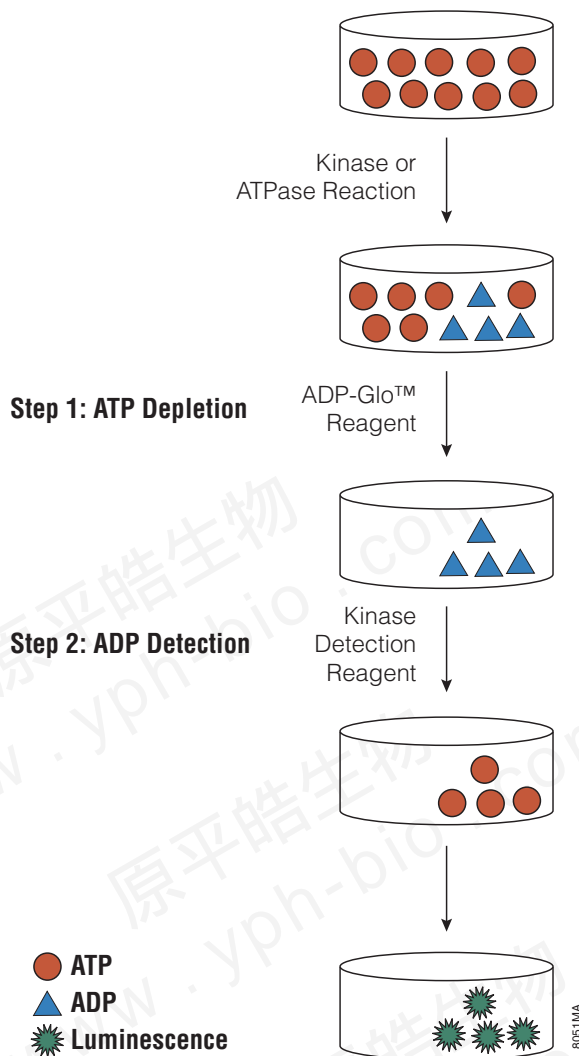
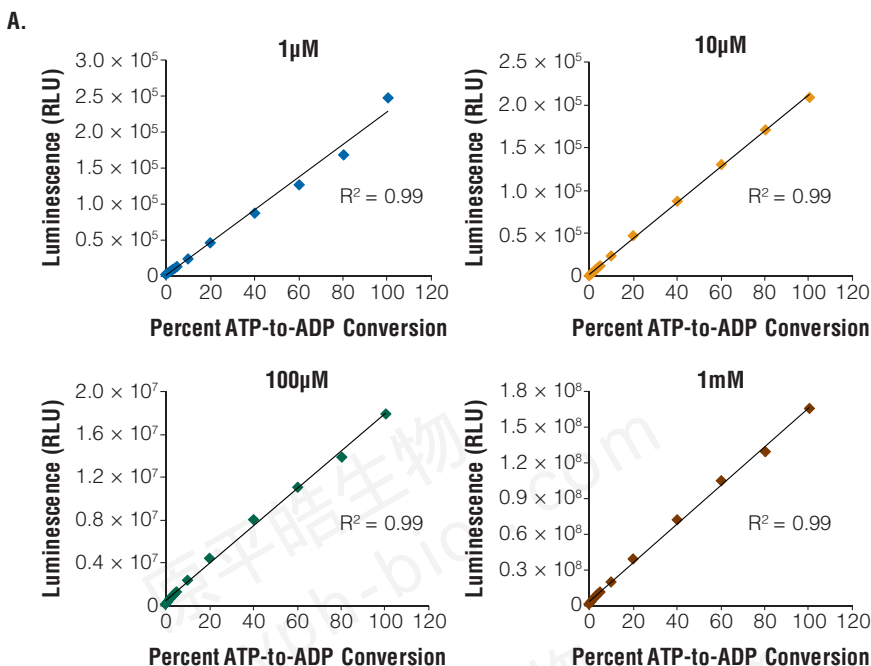


Figure 2. Principle of the ADP-Glo™ Kinase Assay. The assay is performed in two steps: 1) after the kinase or ATPase reaction, ADP-Glo™ Reagent is added to terminate the kinase reaction and deplete the remaining ATP, and 2) the Kinase Detection Reagent is added to convert ADP to ATP and allow the newly synthesized ATP to be measured using a luciferase/luciferin reaction. The light generated correlates to the amount of ADP generated in the kinase or ATPase assay, which is indicative of kinase or ATPase activity.

1. Description (continued)



B.

Signal-to-Background Ratios

		Percent ADP in an ATP + ADP Mixture											
		100	80	60	40	20	10	5	4	3	2	1	0
[ATP + ADP]	1µM	80	54	41	28	15	8	4	4	3	2	2	1
	10µM	135	110	84	57	31	16	8	7	6	4	2	1
	100µM	125	97	77	56	31	17	9	8	6	5	3	1
	1mM	117	91	74	51	28	14	8	7	6	4	2	1

Figure 3. Sensitivity and linearity of the ADP-Glo™ Kinase Assay. Panel A. Four ATP-to-ADP conversion curves were prepared at the indicated ATP+ADP concentrations in 25µl of 1X reaction buffer A (see Section 7 for composition) in a solid white 96-well plate. (Conversion curves are described in Section 3.B.) ADP-Glo™ Kinase Assays were performed using 25µl of ADP-Glo™ Reagent and 50µl of Kinase Detection Reagent at room temperature as described in Section 4.B. Luminescence was recorded using a GloMax® 96 Microplate Luminometer (Cat.# E6501). Values represent the mean of two replicates. There is a linear relationship between the luminescent signal and the amount of ADP in the reaction buffer at all ATP+ADP concentration series tested. Panel B. The ADP-Glo™ Kinase Assay is highly sensitive as shown by the high signal-to-background ratios. The ADP-Glo™ Kinase Assay luminescence was measured 1 hour after adding the Kinase Detection Reagent. To determine signal stability, luminescence was recorded again every hour. The signal is stable over a period of 5 hours generally with <20% increase after 3 hours, while the signal-to-background ratio did not change (data not shown). RLU = relative light units.

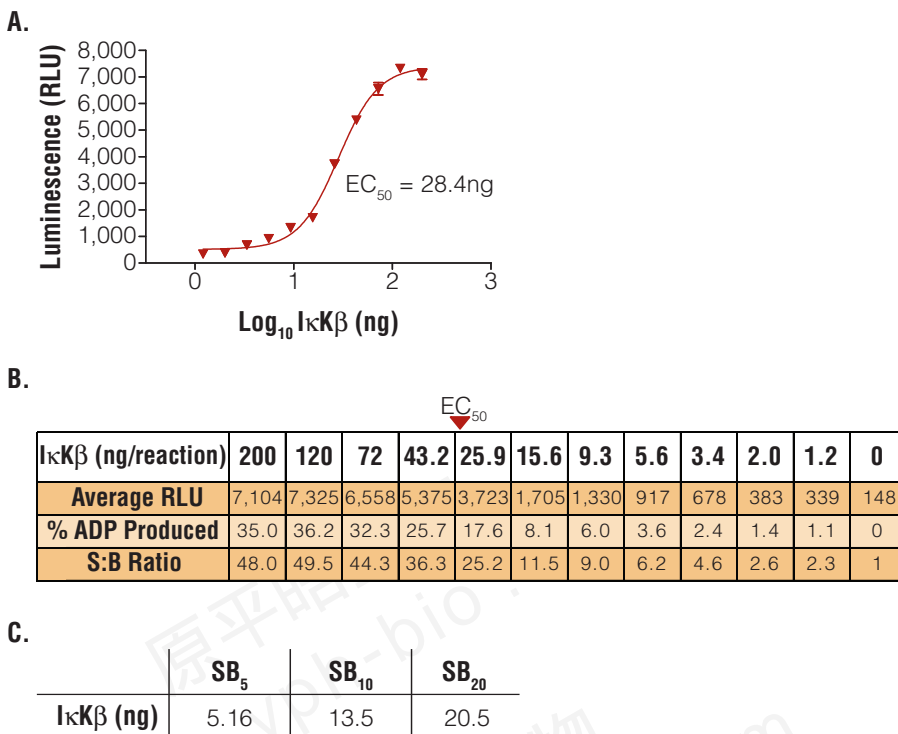


Figure 4. Demonstration of ADP-Glo™ Kinase Assay performance using Inhibitor of κ B kinase β (IκKβ). **Panel A.** The 10 μ l kinase reaction was performed in a 384-well plate in 1X reaction buffer A supplemented with 2mM MnCl₂, 2mM DTT and 100 μ M sodium vanadate using 200 μ M IκKtide peptide (Biomol) as a substrate and 1 μ M ATP at room temperature for 1 hour. ADP was detected as described in Section 4.B using 10 μ l of ADP-Glo™ Reagent and 20 μ l of Kinase Detection Reagent. Curve fitting was performed using GraphPad Prism® sigmoidal dose-response (variable slope) software. **Panel B.** An ATP-to-ADP conversion curve in the 1 μ M series was performed at the same time to correlate the %ADP produced to each RLU value. **Panel C.** Low amounts of enzyme (less than EC₅₀) can be used in the kinase reaction, while maintaining high signal-to-background ratios (S:B Ratio). Data points represent the average of duplicate wells. SB₅, SB₁₀ and SB₂₀ are defined as the amounts of enzyme needed to achieve signal-to-background ratios of 5, 10 and 20, respectively.

1. Description (continued)

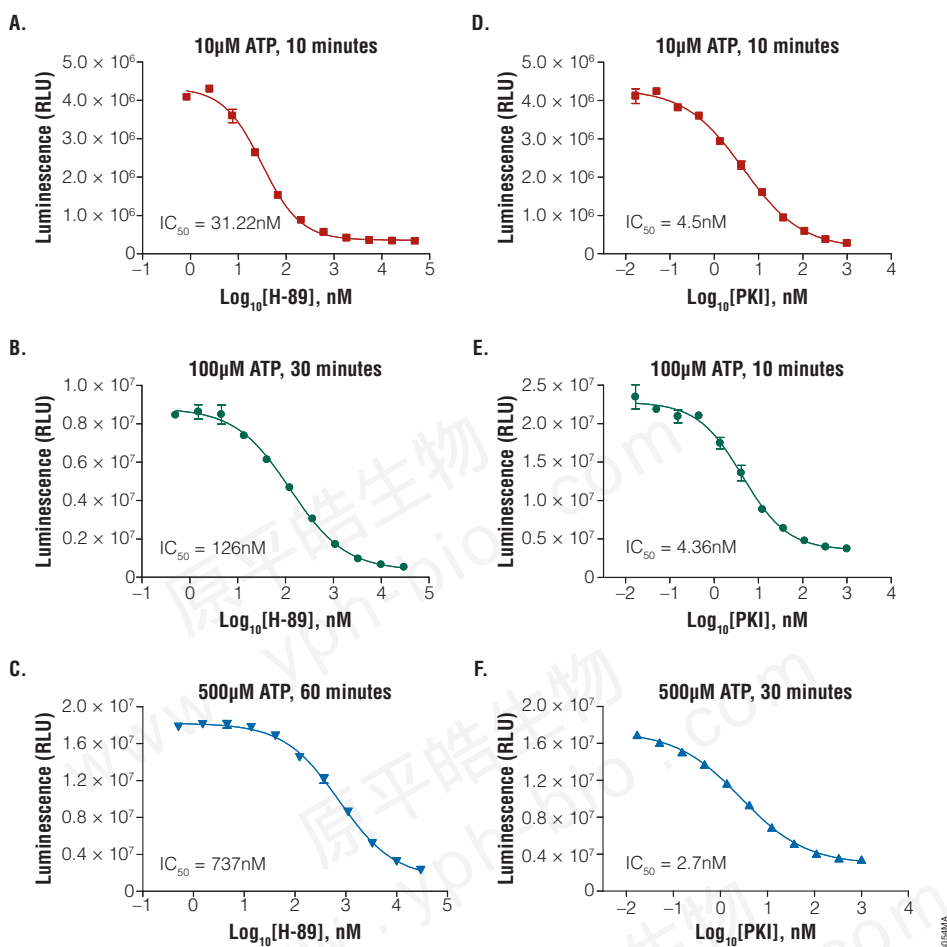


Figure 5. Determining IC₅₀ for ATP competitive and noncompetitive inhibitors.

Titration of the protein kinase A (PKA) noncompetitive inhibitor PKI and competitive inhibitor H-89 were performed in solid white, flat-bottom 96-well plates in a total volume of 50 μ l using 0.1 unit/well PKA, 50 μ M Kemptide (PKA) Peptide Substrate (Cat.# V5601) and the indicated concentration of inhibitor. Reactions were carried out at 10, 100 or 500 μ M ATP for the time listed on each graph. Data points are the average of two determinations, and error bars are \pm S.D. IC₅₀ values for H-89 determined using the ADP-Glo™ Kinase Assay increase with increasing ATP concentration (**Panels A, B and C**). In contrast, the IC₅₀ values for PKI show only a minimal change with increasing ATP concentrations (**Panels D, E and F**). These compare favorably to the IC₅₀ values reported for these compounds in the literature [3nM for PKI (4); 40nM for H-89 at 10 μ M ATP (5)]. Curve fitting was performed using GraphPad Prism® sigmoidal dose-response (variable slope) software.

2. Product Components and Storage Conditions

Product	Size	Cat.#
ADP-Glo™ Kinase Assay	1,000 assays	V9101

The system is sufficient for 1,000 assays if performed in 384-well plates using 5µl, 5µl and 10µl of a kinase reaction, ADP-Glo™ Reagent and Kinase Detection Reagent, respectively, per sample. The system also can be used in 96-well plates using 25µl:25µl:50µl for a total of 200 assays. Includes:

- 5ml ADP-Glo™ Reagent
- 10ml Kinase Detection Buffer
- 1 vial Kinase Detection Substrate (Lyophilized)
- 500µl Ultra Pure ATP, 10mM
- 500µl ADP, 10mM

Product	Size	Cat.#
ADP-Glo™ Kinase Assay	10,000 assays	V9102

The system is sufficient for 10,000 assays if performed in 384-well plates using 5µl, 5µl and 10µl of a kinase reaction, ADP-Glo™ Reagent and Kinase Detection Reagent, respectively, per sample. The system also can be used in 96-well plates using 25µl:25µl:50µl for a total of 2,000 assays. Includes:

- 50ml ADP-Glo™ Reagent
- 100ml Kinase Detection Buffer
- 1 vial Kinase Detection Substrate (Lyophilized)
- 5ml Ultra Pure ATP, 10mM
- 5ml ADP, 10mM

Product	Size	Cat.#
ADP-Glo™ Kinase Assay	100,000 assays	V9103

The system is sufficient for 100,000 assays if performed in 384-well plates using 5µl, 5µl and 10µl of a kinase reaction, ADP-Glo™ Reagent and Kinase Detection Reagent, respectively, per sample. The system also can be used in 96-well plates using 25µl:25µl:50µl for a total of 20,000 assays. Includes:

- 10 × 50ml ADP-Glo™ Reagent
- 10 × 100ml Kinase Detection Buffer
- 10 vials Kinase Detection Substrate (Lyophilized)
- 10 × 5ml Ultra Pure ATP, 10mM
- 10 × 5ml ADP, 10mM

Storage Conditions: Store the system at -20°C. Before use, thaw all components completely at room temperature. Once thawed, mix all components thoroughly before use. Once prepared, dispense Kinase Detection Reagent (Kinase Detection Buffer + Substrate) into aliquots and store at -20°C. Kinase Detection Buffer may form a precipitate when thawed. See Section 3.A for a protocol to dissolve any precipitate. For convenience, ADP-Glo™ Reagent and the Kinase Detection Reagent may be used at room temperature (22°C) for 24 hours without loss of signal.



3. Preparing for the ADP-Glo™ Kinase Assay

Materials to Be Supplied by the User

- solid white, multiwell plate
- multichannel pipette or automated pipetting station
- kinase substrate
- kinase
- luminometer capable of reading multiwell plates
- plate shaker

3.A. Preparing the Kinase Detection Reagent

Kinase Detection Buffer Preparation

The Kinase Detection Buffer may contain a precipitate depending on conditions used for storage and handling. 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 the following steps.

1. Thaw Kinase Detection Buffer at room temperature, and observe for the presence of precipitate.
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. We have shown that the reconstituted reagent remains stable with no loss of signal after several freeze-thaw cycles.

3.B. Generating a Standard Curve for Conversion of ATP to ADP

ADP-Glo™ Kinase Assay can detect small changes in ATP-to-ADP conversion. Whether assaying a low-activity kinase whose ATP turnover rate is low or using a small amount of enzyme that produces a small amount of ADP, a high signal-to-background ratio is obtained with the ADP-Glo™ Kinase Assay (Figure 3). The ADP-Glo™ Kinase Assay produces excellent Z'-factor values (Table 1) as Z' factors greater than 0.5 indicate excellent assay quality (6).

Table 1. Z' Values Produced at Different Percent ADP in an ATP+ADP Mixture Using Two Concentration Series.

ATP-to-ADP Conversion	10μM ATP+ADP	500μM ATP+ADP
1%	0.76	0.65
5%	0.82	0.84
10%	0.90	0.86
20%	0.88	0.92

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 conversion curves represent the amounts of ATP and ADP available in a reaction at the specified conversion percentage (Table 2). 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 (Table 3). The sum of the ATP and ADP concentrations is denoted as “ATP+ADP” in this manual.

Table 2. Percent Conversion of ATP to ADP Represented by the Standard Curve.

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

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.



Use only the provided Ultra Pure ATP when performing the ADP-Glo™ Kinase Assay. Other sources of ATP may contain ADP that could result in high background.

2. Add 90μl of 1X kinase reaction buffer to wells B1-B12, C1-C12 and D1-D12 of a 96-well plate.

3.B. Generating a Standard Curve for Conversion of ATP to ADP (continued)

- Combine the 1mM ATP and 1mM ADP solutions prepared in Step 1 in wells A1–A12 as indicated in Table 3 to simulate the ATP and ADP concentrations at each percent conversion (see Table 2). Mix well. This is the 1mM series.

Table 3. 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


- Dilute the samples in wells A1–A12 by transferring 10μl of the sample in well A1 to well B1, 10μl from well A2 to well B2, etc. Mix well. This is the 100μM series.
- Dilute the samples in wells B1–B12 by transferring 10μl of the sample in well B1 to well C1, 10μl from well B2 to well C2, etc. Mix well. This is the 10μM series.
- Dilute the samples in wells C1–C12 by transferring 10μl of the sample in well C1 to well D1, 10μl from well C2 to well D2, etc. Mix well. This is the 1μM series.
- Transfer the amount needed (5 or 25μl) from the desired ATP+ADP series into separate wells of the new assay plate or the assay plate where the kinase reactions are present.
- Follow the ADP-Glo™ Kinase Assay protocol described in Section 4.B, starting at Step 2.

4. ADP-Glo™ Kinase Assay Protocols

Prior to performing the ADP-Glo™ Kinase Assay, prepare the reagents and ATP+ADP standards as described in Section 3. Calculate the volume of ADP-Glo™ Kinase Assay Reagents required for your experiments, and allow that volume to reach room temperature before use. Return the remaining ADP-Glo™ Reagent and Kinase Detection Reagent to –20°C. For information about optimizing the kinase and kinase substrate concentrations, refer to Section 4.C. The ADP-Glo™ Reagent and the Kinase Detection Reagent may be used at room temperature (22°C) for 24 hours without loss of signal.

4.A. Kinase Reaction Buffer Considerations

The ADP-Glo™ Kinase Assay is designed for use with 1X reaction buffer A (see Section 7 for composition) in the presence of the kinase, ATP and substrate. If protein tyrosine kinase or I κ K β reactions are performed, we recommend supplementing reaction buffer A with 2mM DTT, 2mM MnCl₂ and 100 μ M sodium vanadate. Sphingosine kinase reaction can be performed in 1X reaction buffer A supplemented with 0.5mM DTT.

 If other reaction buffer compositions are preferred, make sure that the MgCl₂ concentration is at least 0.5mM after adding the ADP-Glo™ Reagent to the kinase reaction.

4.B. ADP-Glo™ Kinase Assay Protocol


The ADP-Glo™ Kinase Assay is performed in two steps once the kinase reaction is complete as outlined in Figure 1. For 96-well plates, we recommend 25 μ l of kinase reaction, 25 μ l of ADP-Glo™ Reagent and 50 μ l of Kinase Detection Reagent for a total volume of 100 μ l. For 384-well plates, volumes are reduced fivefold to 5 μ l of kinase reaction, 5 μ l of ADP-Glo™ Reagent and 10 μ l of Kinase Detection Reagent. 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.

 Use the Ultra Pure ATP provided with the ADP-Glo™ Kinase Assay when setting up standard curves and running kinase reactions.

1. Perform a 5 μ l kinase reaction using 1X kinase buffer (e.g., 1X reaction buffer A).

 If the kinase reaction was not run at room temperature, equilibrate the plate to room temperature **before** adding the ADP-Glo™ Reagent.

2. Add 5 μ l of ADP-Glo™ Reagent to stop the kinase reaction and deplete the unconsumed ATP, leaving only ADP and a very low background of ATP.

 The ADP-Glo™ Reagent is effective in terminating the kinase reaction; therefore, there is no need to add an inhibitor to terminate the kinase reaction. However, although not recommended, if the protocol is modified to add a kinase-termination reagent, do not use a magnesium-chelating agent such as EDTA because the ADP-Glo™ Assay requires the presence of magnesium.

Note: The final Mg²⁺ concentration must be at least 0.5mM.

3. Incubate at room temperature for 40 minutes.

4. Add 10 μ l of Kinase Detection Reagent to convert ADP to ATP and introduce luciferase and luciferin to detect ATP.

4.B. ADP-Glo™ Kinase Assay Protocol (continued)

5. Incubate at room temperature for 30–60 minutes, depending on the ATP concentration used in the kinase reaction (see Table 4).

Table 4. 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.

4.C. Optimizing Kinase Reaction Conditions

For optimal performance when using the ADP-Glo™ Kinase Assay, optimize the amounts of kinase and kinase substrate in the kinase reaction. If the amount of kinase or kinase substrate has been determined, proceed to Section 4.B.

When screening for kinase inhibitors, use an ATP concentration of 10μM or less to preferentially identify ATP competitive inhibitors. To preferentially identify ATP-noncompetitive inhibitors, use a higher ATP concentration (up to 1mM). Optimize enzyme and substrate concentrations, reaction temperature and incubation time whenever the ATP concentration is changed.

Notes:

1. Use **only** the provided Ultra Pure ATP when performing the ADP-Glo™ Kinase Assay. Other sources of ATP may contain ADP that could result in high background.
2. We recommend optimizing the kinase reaction conditions at room temperature to ensure uniform temperature across the plate during the ADP-Glo™ Kinase Assay.


Determining Optimal Substrate Concentration

1. Make serial twofold dilutions of kinase substrate in 1X kinase reaction buffer across the plate using as much kinase as is practical and the desired concentration of ATP (up to 1mM). As a control, perform the same titration without kinase. Mix the plate, and incubate for the desired amount of time.
2. Follow the ADP-Glo™ Kinase Assay protocol described in Section 4.B, starting at Step 2.
3. Record luminescence.

Note: The optimal kinase substrate concentration will result in the largest difference in luminescence between kinase reaction wells and wells that do not contain kinase.

Determining the Optimal Amount of Kinase

1. Make serial twofold dilutions of kinase in 1X kinase reaction buffer across the plate using the desired amount of ATP (up to 1mM) and kinase substrate (as determined previously). Mix the plate, and incubate for the desired amount of time.

 We recommend optimizing the kinase reaction conditions at room temperature to ensure uniform temperature across the plate during the ADP-Glo™ Assay.

2. Follow the ADP-Glo™ Kinase Assay protocol described in Section 4.B, starting at Step 2.
3. Record luminescence.

Note: The optimal amount of kinase to use in subsequent compound screens and IC₅₀ determinations is the amount that produces luminescence within the linear range of the kinase titration curve and generates an adequate signal-to-background ratio.

Because the ADP-Glo™ Kinase Assay is very sensitive, it can detect a very small amount of ADP with a high signal-to-background ratio. A small amount of enzyme that produces low conversion of ATP to ADP is sufficient for use with the ADP-Glo™ Kinase Assay due to the high signal-to-background ratio generated. Figure 4 shows that the amount of ADP produced with a small amount of enzyme results in a signal-to-background ratio (SB) of 5, 10 or 20 (SB₅, SB₁₀ and SB₂₀, respectively).

4.D. Screening for Inhibitors Using the ADP-Glo™ Kinase Assay

The following protocol is an example; the actual volumes used can be adjusted as needed. The volume of the ADP-Glo™ Reagent used should equal the volume of the kinase reaction, and the volume of the Kinase Detection Reagent should be double the amount of the kinase reaction. The volumes provided are intended for a 384-well plate (5µl:5µl:10µl ratio); to perform the assay in a 96-well plate, increase the volumes fivefold.

1. Add 1µl of test compound to each well except for 16 control wells, which receive 1µl of test compound vehicle.
2. Add 2µl of 1X kinase reaction buffer containing 2.5X the optimal concentration of kinase, but no kinase substrate, to eight of the control wells.
3. Add 2µl of 1X kinase reaction buffer containing 2.5X the optimal concentrations of kinase and kinase substrate to all remaining wells.
4. Add 2µl of 1X kinase reaction buffer containing 2.5X the desired concentration of ATP (up to 1mM) to all wells.

4.D. Screening for Inhibitors Using the ADP-Glo™ Kinase Assay (continued)

- Mix the plate, and incubate for the desired amount of time.

! We recommend optimizing the kinase reaction conditions at room temperature to ensure uniform temperature across the plate during the ADP-Glo™ Assay.

- Follow the ADP-Glo™ Kinase Assay protocol described in Section 4.B, starting at Step 2.
- Record luminescence.

Note: Instrument settings depend on the manufacturer. An integration time of 0.25–1 second per well should serve as a guideline.

4.E. Determining IC₅₀ Values of Kinase Inhibitors

The following protocol is designed for a 96-well plate using a 25µl:25µl:50µl ratio. To perform the assay in a 384-well plate, reduce volumes fivefold. 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.

- Add 10µl of 1X kinase reaction buffer to each well in columns 2 through 12.
- Add 20µl of the inhibitor diluted to the highest concentration desired in 1X kinase reaction buffer to the wells in column 1.
- Transfer 10µl from the wells in column 1 to column 2. Mix well. Continue to make twofold serial dilutions across the plate, mixing well before each transfer. Discard the 10µl removed from the last column.
- Add 10µl of 1X kinase reaction buffer containing 2.5X the optimal concentration of enzyme to all wells.
- Add 5µl of 1X kinase reaction buffer containing 5X the optimal concentration of ATP and 5X the optimal concentration of substrate to all wells.

! Use only the provided Ultra Pure ATP when performing the ADP-Glo™ Kinase Assay. Other sources of ATP may contain ADP that could result in high background.

- Mix the plate, and incubate for the optimal amount of time.

! We recommend optimizing the kinase reaction conditions so that the reaction can be performed at room temperature to avoid formation of temperature gradients.

Note: If the kinase reaction was not run at room temperature, equilibrate the plate to room temperature **before** adding the ADP-Glo™ Reagent.

- Follow the ADP-Glo™ Kinase Assay protocol described in Section 4.B, starting at Step 2.
- Record luminescence.

Note: Instrument settings depend on the manufacturer. An integration time of 0.25–1 second per well should serve as a guideline.

5. General Considerations

Temperature: The intensity and stability of the luminescent signal from the ADP-Glo™ Kinase Assay depends on the rate of the luciferase reaction. Environmental factors that affect the rate of the luciferase reaction will result in a change in the intensity of light output and stability of the luminescent signal. Temperature is one factor that affects the rate of this enzymatic assay and thus the light output. For consistent results, equilibrate assay plates and reagent to room temperature before adding the ADP-Glo™ Kinase Assay reagents. Insufficient equilibration may result in a temperature gradient between the wells in the center and at the edge of the plate and therefore variability across the plate.

Solvents and Other Chemicals: The chemical environment in which the first or second step of the ADP-Glo™ Kinase Assay is performed will affect the enzymatic rates and thus luminescence intensity. We recommend a pH of 7-8 for the kinase buffer. Some vehicles used to resuspend the various test compounds or reagents used in the kinase reaction buffer may interfere with the luciferase reaction and thus affect the light output of the assay. Various chemicals were shown to be compatible with or tolerated by the ADP-Glo™ Kinase Assay (Table 5). Interference with the luciferase reaction can be detected by assaying a parallel set of control wells without kinase or kinase substrate and using ADP as a substrate.

Table 5. Solvents and Chemicals Compatible with the ADP-Glo™ Kinase Assay.

Chemical	Maximum Concentration Tolerated
NaCl	200mM
CaCl ₂	2.5mM
Calmodulin	5µM
DTT	10mM
MgCl ₂	50mM
MnCl ₂	5mM
DMSO	5%
Sodium orthovanadate	500µM
Tergitol-NP-9	0.1%
Tween®-20	0.2%
Triton® X-100	0.2%

Plate Recommendations: We recommend using standard solid white, multiwell plates suitable for luminescence measurements. Please contact your luminometer instrument manufacturer to find out which plates will work best for your particular instrument.



5. General Considerations (continued)

Testing for Compounds that Interfere with the ADP-Glo™ Kinase Assay:

Test compounds that inhibit only the kinase will result in lower luminescence compared to vehicle-only controls and are easily distinguishable from compounds that inhibit other components of the assay. Test compounds that inhibit other components of the assay either alone or together with the kinase might increase, decrease or have no effect on the luminescent signal, depending on the level of inhibition of the kinase, luciferase or other enzyme components. To test hits from a kinase screen for the possibility of chemical interference with enzymatic depletion of ATP or generation of the luminescent signal, set up mock reactions without kinase but with all other assay components present, including a mixture of ADP and ATP to mimic the kinase reaction results. Add the appropriate concentration of test compound (usually 10 μ M) or vehicle control (e.g., 1% DMSO) to mock reactions without kinase present but containing final concentrations of 2 μ M ADP and 8 μ M ATP (as described for generating a standard curve for conversion of ATP to ADP, Section 3.B). A test compound that affects assay performance would alter luminescence by greater than 20% compared to vehicle control reactions without test compounds. The results from testing 30 ATP competitive inhibitors from different chemical classes showed that none interfered with the ADP-Glo™ Kinase Assay (7). Test compounds that inhibit luciferase may result in false hits. However, the unique combination of Ultra-Glo™ Recombinant Luciferase and proprietary buffer compositions of the ADP-Glo™ Kinase Assay significantly reduces the number of false hits (1,3).

6. References

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7. Composition of Buffer

1X Kinase Reaction Buffer A

40mM	Tris (pH 7.5)
20mM	MgCl ₂
0.1mg/ml	BSA

8. Related Products

Protein Kinase Assay Systems

Product	Size	Cat.#
Kinase-Glo [®] Luminescent Kinase Assay	10ml	V6711
	10 × 10ml	V6712
	100ml	V6713
	10 × 100ml	V6714
Kinase-Glo [®] Plus Luminescent Kinase Assay	10ml	V3771
	10 × 10ml	V3772
	100ml	V3773
	10 × 100ml	V3774
Kinase-Glo [®] Max Luminescent Kinase Assay	10ml	V6071
	10 × 10ml	V6072
	100ml	V6073
	10 × 100ml	V6074
ProFluor [®] PKA Assay	4 plate	V1240
	8 plate	V1241
ProFluor [®] Src-Family Kinase Assay	4 plate	V1270
	8 plate	V1271
PepTag [®] Non-Radioactive PKC Assay	120 reactions	V5330
PepTag [®] Non-Radioactive cAMP-Dependent Protein Kinase Assay	120 reactions	V5340

SignaTECT[®] Protein Kinase Assay Systems

Product	Size	Cat.#
SignaTECT [®] cAMP-Dependent Protein Kinase (PKA) Assay System	96 reactions	V7480
SignaTECT [®] Protein Kinase C (PKC) Assay System	96 reactions	V7470
SignaTECT [®] Protein Tyrosine Kinase (PTK) Assay System	96 reactions	V6480
SignaTECT [®] Calcium/Calmodulin-Dependent Protein Kinase (CaM KII) Assay System	96 reactions	V8161
SignaTECT [®] DNA-Dependent Protein Kinase Assay System	96 reactions	V7870
SignaTECT [®] cdc2 Protein Kinase Assay System	96 reactions	V6430



8. Related Products (continued)

SAM²® Biotin Capture Membranes

Product	Size	Cat.#
SAM ² ® Biotin Capture Membrane	96 samples	V2861
	7.6 × 10.9cm	V7861
SAM ² ® 96 Biotin Capture Plate	96-well plate	V7541
	5 × 96-well plates	V7542

For Laboratory Use.

Purified Kinases

Product	Size	Cat.#
cAMP-Dependent Protein Kinase, Catalytic Subunit	2,500u	V5161
Protein Kinase C	1µg	V5261
EGF Receptor	10u	V5551
DNA-Dependent Protein Kinase	2,500u	V5811
Casein Kinase I	100u	V5631
Casein Kinase II	100u	V5621

^(a)Patents Pending.

^(b)Australian Pat. No. 2003268489 and other patents pending.

^(c)U.S. Pat. Nos. 6,602,677 and 7,241,584, Australian Pat. Nos. 754312 and 785294, European Pat. No. 1131441 and other patents pending.

^(d)The method of recombinant expression of *Coleoptera* luciferase is covered by U.S. Pat. Nos. 5,583,024, 5,674,713 and 5,700,673.

^(e)Licensed from Lonza Nottingham Ltd. under U.S. Pat. Nos. 6,599,711 and 6,911,319 and other pending and issued patents.

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Promega Corporation • 2800 Woods Hollow Road
Madison, WI 53711-5399 USA • Phone 608-274-4330



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