Technical Bulletin

Kinase-Glo® Luminescent Kinase Assay Platform

INSTRUCTIONS FOR USE OF PRODUCTS V6711, V6712, V6713, V6714, V3771, V3772, V3773, V3774, V6071, V6072, V6073 AND V6074.

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Kinase-Glo® Luminescent Kinase Assay Platform

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1. Description

The Kinase-Glo® Luminescent Kinase Assay Platform provides a homogeneous, high-throughput screening method for measuring kinase activity by quantitating the amount of ATP remaining in solution following a kinase reaction. The Kinase-Glo® Platform consists of three assay formats: the Kinase-Glo® Assay[a,b,c], which is used to monitor kinase activity using up to 10µM ATP; the Kinase-Glo® Plus Assay[a,c], which is used for assays requiring higher ATP concentrations (up to 100µM); and the Kinase-Glo® Max Assay[a,c], which is used for assays requiring up to 500µM ATP. The Kinase-Glo® Assays are performed in a single well of a multiwell plate by adding a volume of Kinase-Glo® Reagent equal to the volume of a completed kinase reaction and measuring luminescence (Figures 1 and 2). The luminescent signal is correlated with the amount of ATP present (Figure 3) and is inversely correlated with the amount of kinase activity (Figure 4).

These assays can be performed with virtually any kinase and substrate combination and do not require radioactively labeled components. The kinase substrate can be a peptide, protein, lipid or sugar. The Kinase-Glo® Reagents rely 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 luminescent signal, which is produced by the luciferase reaction shown in Figure 2, has a half-life greater than five hours (Figure 5). This extended half-life eliminates the need for a luminometer with reagent injectors and allows batch-mode processing of multiple plates. In addition, the unique combination of Ultra-Glo™ Luciferase and a proprietary buffer formulation results in luminescence that is much less susceptible to interference from library compounds than other luciferase-based ATP detection reagents (1–3). The Kinase-Glo® Luminescent Kinase Assays easily detect known kinase inhibitors and produce excellent Z´-factor values (Figure 6). In addition to providing IC_{50} values comparable to those reported in the literature, these assays can be used to distinguish between ATP-competitive and ATP-noncompetitive inhibitors (Figures 7 and 8).

Advantages of the Kinase-Glo® Assay Platform:

- **Use higher ATP concentrations:** Linear response up to 500µM ATP.
- **Use any kinase and kinase-substrate combination**, including peptide, protein, lipid and sugar substrates.
- **Perform the assay without substrate modifications.**
- **Perform batch plate-processing:** Highly stable luminescent signal, with over 50% signal remaining at 5 hours.
- **Distinguish between ATP-competitive and ATP-noncompetitive kinase inhibitors.**
- **Screen large numbers of library compounds** with this fast, homogeneous, non-radioactive assay.
- **Obtain reliable, reproducible data:** Z´-factor values routinely >0.7.
- **Reduce false hits**: the combination of Ultra-Glo™ Luciferase and the proprietary buffer formulation reduces the incidence of false hits.

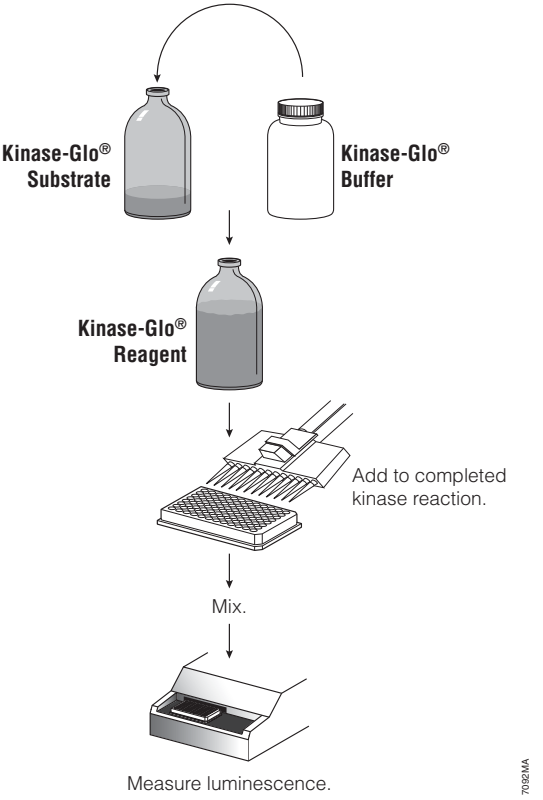

Figure 1. Schematic representation of the Kinase-Glo® Luminescent Kinase Assay protocols.

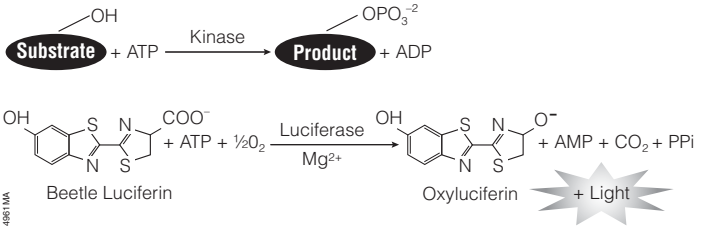

Figure 2. The Kinase-Glo® Assay reactions. The kinase reaction is conducted under the appropriate conditions. ATP remaining at the time that the reagent is added is used as a substrate by the Ultra-Glo™ Luciferase to catalyze the mono-oxygenation of luciferin. The luciferase reaction produces one photon of light per turnover. Luminescence is inversely related to kinase activity.

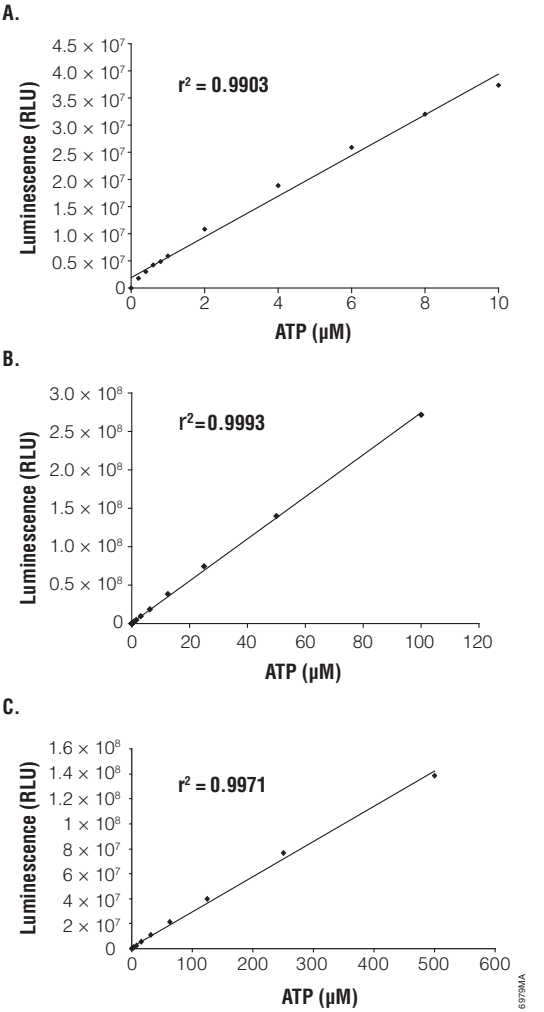

Figure 3. Luminescence correlates with amount of ATP. A direct relationship exists between the luminescence measured with the Kinase-Glo® Reagent and the amount of ATP. Serial dilutions of ATP were made in a solid white, 96-well plate in 50µl kinase reaction buffer (40mM Tris [pH 7.5], 20mM $MgCl_2$, and 0.1mg/ml BSA). An equal volume of the appropriate Kinase-Glo® Reagent was added to each well, incubated at room temperature, and then 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 ATP in the kinase reaction buffer from 0–10µM using the Kinase-Glo® Assay (**Panel A**, $r^2 = 0.9903$); 0–100µM using the Kinase-Glo® Plus Assay (**Panel B**, $r^2 = 0.9993$); and 0–500µM using the Kinase-Glo® Max Assay (**Panel C**, $r^2 = 0.9971$).

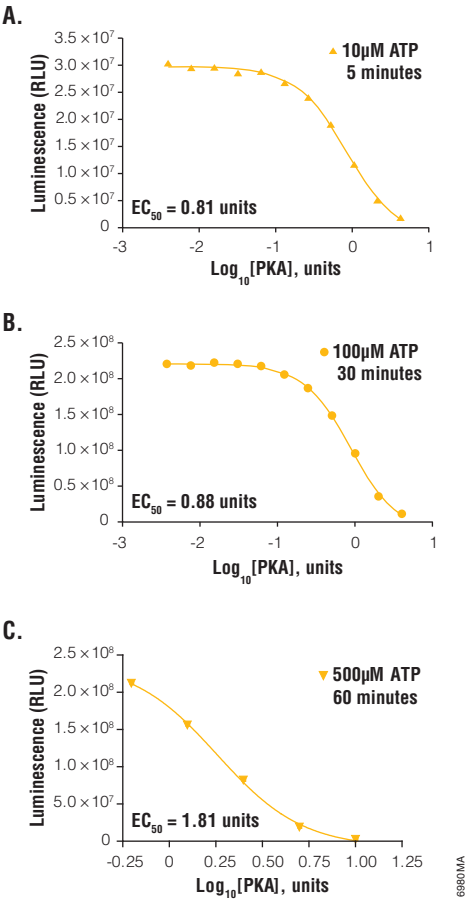

Figure 4. Luminescence is inversely correlated with kinase activity. An inverse relationship exists between luminescence measured with the Kinase-Glo® Reagent and the amount of kinase activity. Serial twofold dilutions of kinase were made in solid white, 96-well plates in 50µl kinase reaction buffer. Kinase reactions containing 40mM Tris-HCl (pH 7.5), 0.1 mg/ml BSA, 20mM $MgCl_2$ were performed at 10µM ATP, 50µM Kemptide substrate (Cat.# V5601) with 1 unit of PKA for 5 minutes using the Kinase-Glo® Assay (**Panel A**), 100µM ATP and 500µM Kemptide substrate and 1.5 units of PKA for 30 minutes using the Kinase-Glo® Plus Assay (**Panel B**), or 500µM ATP and 500µM Kemptide and 1.5 units of PKA for 60 minutes using the Kinase-Glo® Max Assay (**Panel C**). Following the kinase reaction, an equal volume of Kinase Glo® Max Reagent was added. Luminescence was recorded on a GloMax® 96 Microplate Luminometer (Cat.# E6501) after ten minutes. Curve fitting was performed using GraphPad Prism® sigmoidal dose-response (variable slope) software.

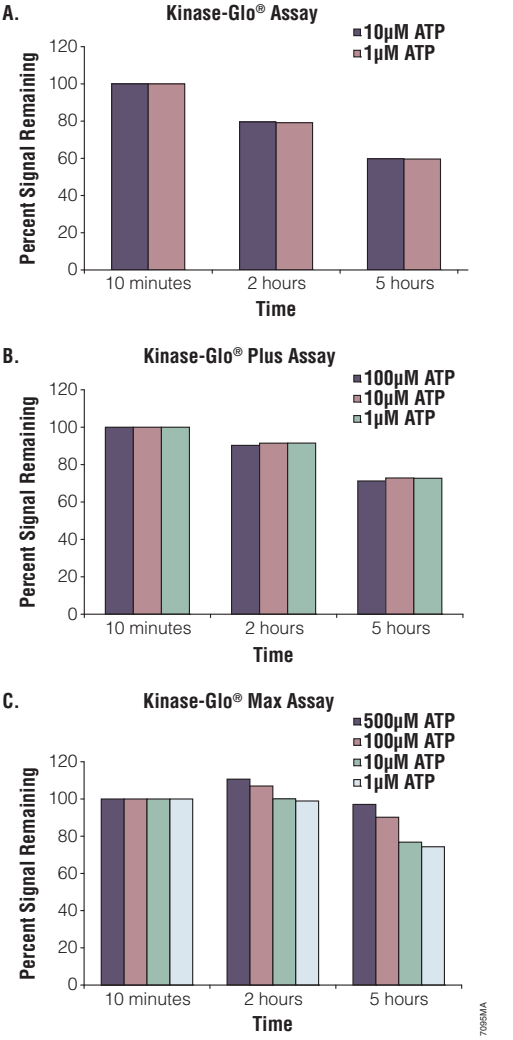

Figure 5. Extended luminescent glow-type signal. Signal stability obtained with Kinase-Glo® (**Panel A**), Kinase-Glo® Plus (**Panel B**), and Kinase-Glo® Max (**Panel C**) Reagents in a solid white, 96-well plate (n = 24). Aliquots of 25µl of Kinase reaction buffer (40mM Tris [pH 7.5], 20mM $MgCl_2$, and 0.1mg/ml BSA) containing 1.0µM, 10µM, 100µM, or 500µM ATP were added, and equal volumes of Kinase-Glo®, Kinase-Glo® Plus, or Kinase-Glo® Max Reagents were added and incubated 10 minutes. Luminescence was recorded using a GloMax® 96 Microplate Luminometer (Cat.# E6501). Luminescence was measured again at the indicated times. The signal decreased less than 25% in two hours and less than 50% in five hours. RLU = relative light units.

2. Product Components and Storage Conditions

2.A. Kinase-Glo® Max Luminescent Kinase Assay

Product	Size	Cat.#
Kinase-Glo® Max Luminescent Kinase Assay	10ml	V6071

10ml of Kinase-Glo® Max Reagent is sufficient for 200 assays at 50µl/assay in 96-well plates or 1,000 assays at 10µl/assay in 384-well plates. Includes:

- 10ml Kinase-Glo® Max Buffer
- 1 vial Kinase-Glo® Max Substrate (lyophilized)

Product	Size	Cat.#
Kinase-Glo® Max Luminescent Kinase Assay	10 × 10ml	V6072

10ml of Kinase-Glo® Max Reagent is sufficient for 200 assays at 50µl/assay in 96-well plates or 1,000 assays at 10µl/assay in 384-well plates (2,000 to 10,000 total assays). Includes:

- 10 × 10ml Kinase-Glo® Max Buffer
- 10 vials Kinase-Glo® Max Substrate (lyophilized)

Product	Size	Cat.#
Kinase-Glo® Max Luminescent Kinase Assay	100ml	V6073

100ml of Kinase-Glo® Max Reagent is sufficient for 2,000 assays at 50µl/assay in 96-well plates or 10,000 assays at 10µl/assay in 384-well plates. Includes:

- 100ml Kinase-Glo® Max Buffer
- 1 vial Kinase-Glo® Max Substrate (lyophilized)

Product	Size	Cat.#
Kinase-Glo® Max Luminescent Kinase Assay	10 × 100ml	V6074

100ml of Kinase-Glo® Max Reagent is sufficient for 2,000 assays at 50µl/assay in 96-well plates or 10,000 assays at 10µl/assay in 384-well plates (20,000 to 100,000 total assays). Includes:

- 10 × 100ml Kinase-Glo® Max Buffer
- 10 vials Kinase-Glo® Max Substrate (lyophilized)

Storage Conditions: Store the Buffers and the lyophilized Substrates at –20°C.

2. Product Components and Storage Conditions (continued)

2.B. Kinase-Glo® Plus Luminescent Kinase Assay

Product	Size	Cat.#
Kinase-Glo® Plus Luminescent Kinase Assay	10ml	V3771

10ml of Kinase-Glo® Plus Reagent is sufficient for 200 assays at 50µl/assay in 96-well plates or 1,000 assays at 10µl/assay in 384-well plates. Includes:

- 10ml Kinase-Glo® Plus Buffer
- 1 vial Kinase-Glo® Plus Substrate (lyophilized)

Product	Size	Cat.#
Kinase-Glo® Plus Luminescent Kinase Assay	10 × 10ml	V3772

10ml of Kinase-Glo® Plus Reagent is sufficient for 200 assays at 50µl/assay in 96-well plates or 1,000 assays at 10µl/assay in 384-well plates (2,000 to 10,000 total assays). Includes:

- 10 × 10ml Kinase-Glo® Plus Buffer
- 10 vials Kinase-Glo® Plus Substrate (lyophilized)

Product	Size	Cat.#
Kinase-Glo® Plus Luminescent Kinase Assay	100ml	V3773

100ml of Kinase-Glo® Plus Reagent is sufficient for 2,000 assays at 50µl/assay in 96-well plates or 10,000 assays at 10µl/assay in 384-well plates. Includes:

- 100ml Kinase-Glo® Plus Buffer
- 1 vial Kinase-Glo® Plus Substrate (lyophilized)

Product	Size	Cat.#
Kinase-Glo® Plus Luminescent Kinase Assay	10 × 100ml	V3774

100ml of Kinase-Glo® Plus Reagent is sufficient for 2,000 assays at 50µl/assay in 96-well plates or 10,000 assays at 10µl/assay in 384-well plates (20,000 to 100,000 total assays). Includes:

- 10 × 100ml Kinase-Glo® Plus Buffer
- 10 vials Kinase-Glo® Plus Substrate (lyophilized)

Storage Conditions: Store the Buffers and the lyophilized Substrates at –20°C.

2.C. Kinase-Glo® Luminescent Kinase Assay

Product	Size	Cat.#
Kinase-Glo® Luminescent Kinase Assay	10ml	V6711

10ml of Kinase-Glo® Reagent is sufficient for 200 assays at 50µl/assay in 96-well plates or 1,000 assays at 10µl/assay in 384-well plates. Includes:

- 10ml Kinase-Glo® Buffer
- 1 vial Kinase-Glo® Substrate (lyophilized)

Product	Size	Cat.#
Kinase-Glo® Luminescent Kinase Assay	10 × 10ml	V6712

10ml of Kinase-Glo® Reagent is sufficient for 200 assays at 50µl/assay in 96-well plates or 1,000 assays at 10µl/assay in 384-well plates (2,000 to 10,000 total assays). Includes:

- 10 × 10ml Kinase-Glo® Buffer
- 10 vials Kinase-Glo® Substrate (lyophilized)

Product	Size	Cat.#
Kinase-Glo® Luminescent Kinase Assay	100ml	V6713

100ml of Kinase-Glo® Reagent is sufficient for 2,000 assays at 50µl/assay in 96-well plates or 10,000 assays at 10µl/assay in 384-well plates. Includes:

- 100ml Kinase-Glo® Buffer
- 1 vial Kinase-Glo® Substrate (lyophilized)

Product	Size	Cat.#
Kinase-Glo® Luminescent Kinase Assay	10 × 100ml	V6714

100ml of Kinase-Glo® Reagent is sufficient for 2,000 assays at 50µl/assay in 96-well plates or 10,000 assays at 10µl/assay in 384-well plates (20,000 to 100,000 total assays). Includes:

- 10 × 100ml Kinase-Glo® Buffer
- 10 vials Kinase-Glo® Substrate (lyophilized)

Storage Conditions: Store the Buffers and the lyophilized Substrates at –20°C.

3. Protocols for Kinase-Glo® Assays

Materials to Be Supplied by the User

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

3.A. Buffer Preparation

The Buffer may contain a precipitate depending on conditions used for storage and handling. There is no observed change in performance of any of the Kinase-Glo® Assays whether or not the buffer contains a precipitate. However, the precipitate may be removed or solubilized to avoid clogging pipette tips by performing the following steps.

1. Thaw the Buffer at room temperature or 37°C and observe for presence of precipitate.
2. If precipitate is present, incubate the Buffer at 37°C with constant swirling. This procedure usually dissolves the crystals in less than 1 hour.

   Note: Incubation of the Buffer for up to 4 hours at 37°C does not affect its performance.
3. Alternatively, the precipitate can be removed from the Kinase-Glo® Buffer using a centrifugation step or carefully pipetting off the supernatant.
4. For convenience, Kinase-Glo® Buffer may be thawed and stored at room temperature for up to 48 hours. If precipitation is observed, follow the directions above.

3.B. Reagent Preparation

1. Equilibrate the Buffer and Substrate to **room temperature** before use.
2. Transfer the entire volume of the Buffer into the amber bottle containing the Substrate to reconstitute the lyophilized luciferase/substrate mixture. This forms the Kinase-Glo® Reagent.
3. Mix by gently vortexing, swirling or by inverting the contents to obtain a homogeneous solution. The Kinase-Glo® Substrate should go into solution easily in less than one minute.
4. Kinase-Glo® 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 ten freeze-thaw cycles in assays using ATP concentrations ranging up to 500µM and the appropriate Kinase-Glo® Assay.

3.C. Optimizing Kinase Reaction Conditions

For best performance when using the Kinase-Glo® Reagent, optimize the kinase reaction conditions with respect to the amount of kinase and kinase substrate. If you have predetermined for either the amount of kinase or kinase substrate, proceed to the appropriate section of this protocol.

For 96-well plates, we recommend a 50µl kinase reaction and 50µl Kinase-Glo® Reagent for a total volume of 100µl. For 384-well plates, volumes may be reduced fivefold to a 10µl kinase reaction and 10µl Kinase-Glo® Reagent. Other volumes may be used, provided the 1:1 ratio of kinase reaction volume to Kinase-Glo® Reagent volume is maintained.

To bias the assay toward ATP-competitive inhibitors use lower concentrations of ATP (10µM or less). To bias the assay toward ATP-noncompetitive inhibitors, use a higher concentration of ATP and use the Kinase-Glo® Plus Assay (up to 100µM) or Kinase-Glo® Max Assay (up to 500µM). Optimize the assay with regard to enzyme, substrate, temperature and incubation time whenever ATP concentration is changed.

We strongly advise optimizing the kinase reaction conditions so that the reaction can be run at room temperature to avoid formation of temperature gradients.

Determining Optimal Substrate Concentration

1. Make twofold serial dilutions of kinase substrate across the plate using as much kinase as practical and the desired concentration of ATP. As a control, perform the same titration without kinase. Mix contents of plate and incubate for the appropriate amount of time.

   Note: If the kinase reaction was not run at room temperature, equilibrate the plate to room temperature BEFORE adding Kinase-Glo® Reagent.

2. To each well, add a volume of the appropriate Kinase-Glo® Reagent format equal to the volume of the kinase reaction.

   Note: Choice of reagent will depend on the ATP concentration in the assay.

3. Mix the plate and incubate at room temperature for 10 minutes to stabilize the luminescent signal. The long half-life of the Kinase-Glo® signal allows plates to be left longer at room temperature before reading (Figure 5), if desired.

4. 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. The optimal kinase substrate concentration will result in the largest change in luminescence when comparing kinase reaction wells to wells that do not contain kinase.

3.C. Optimizing Kinase Reaction Conditions (continued)

Determining the Optimum Amount of Kinase

1. Make serial twofold dilutions of kinase across the plate using the desired amount of ATP and kinase substrate (as determined previously). Mix contents of plate and incubate for appropriate amount of time.

   We strongly advise optimizing the kinase reaction conditions so that the reaction can be run 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 Kinase-Glo® Reagent.

2. To each well, add a volume of the appropriate Kinase-Glo® Reagent equal to the volume of the kinase reaction. Choice of reagent will depend on the ATP concentration in the assay.

3. Mix the plate and incubate at room temperature for 10 minutes to stabilize luminescence signal. The long half-life of the Kinase-Glo® signal allows plates to be left longer at room temperature before reading, if desired.

4. 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. The optimal amount of kinase to use in subsequent compound screens and IC_{50} determinations should be an amount that produces luminescence values in the linear range of the kinase titration curve.

3.D. Determining Z´ Factor Values

The following is a suggested protocol; the actual volumes used can be adjusted as needed. The main consideration is that the volume of Kinase-Glo® Reagent used should equal the volume of the kinase reaction. This protocol is for 384-well plates. Scaling for 96- or 1536-well plates can be accomplished by adjusting the volumes in each step proportionally. See Section 3.C.

1. Add 5µl of reaction mixture containing 1X kinase reaction buffer and 2X the optimal amount of kinase and kinase substrate to each well in Rows A–D.

2. Prepare the reaction mixture described in Step 1, omitting the kinase or kinase substrate. Add 5µl to each well in Rows E through H.

3. Add 5µl of reaction mixture containing 2X the desired amount of ATP in 1X kinase reaction buffer to all wells.

4. Mix the plate and incubate for the optimum amount of time.

   We strongly advise optimizing the kinase reaction conditions so the reaction can be run 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 Kinase-Glo® Reagent.

5. To each well, add 10µl of the appropriate Kinase-Glo® Reagent . Choice of reagent will depend on ATP concentration in the reaction. See Section 3.C.
6. Mix the plate and incubate for 10 minutes at room temperature. The long half-life of the Kinase-Glo® signal allows plates to be left longer at room temperature before reading, if desired.
7. Record luminescence. Figure 6 presents results from a typical Z´-factor determination.

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

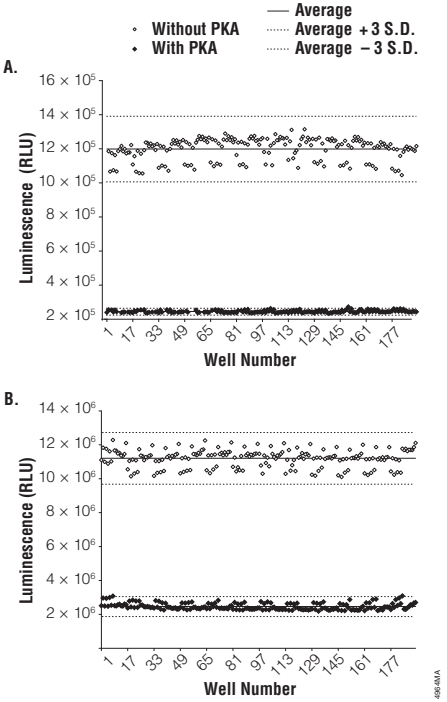

Figure 6. Determining Z´-factor values. Results of Z´-factor analysis (4) in a solid white 384-well, flat-bottom plate. **Panel A.** (Kinase-Glo® Plus Assay) Reactions were performed in solid white, 96-well plates in 50µl kinase reaction buffer (40mM Tris [pH 7.5], 20mM $MgCl_2$, 0.1mg BSA) containing 50µM Kemptide Substrate with 0.2 units/well PKA and 10µM ATP and 5 minutes at room temperature (solid symbols) or without PKA (open symbols). **Panel B.** (Kinase-Glo® Max Assay) PKA was diluted in 50µl kinase reaction buffer (40mM Tris [pH 7.5], 20mM $MgCl_2$, 0.1mg/ml BSA) containing 500µM Kemptide Substrate using 0.2 units/well PKA and 100µM ATP for 30 minutes at room temperature (solid symbols) or without PKA (open symbols). Final volumes for the 384-well plate assays were 20µl. Solid lines indicate the mean, and the dotted lines indicate ± 3 S.D. Z´-factor values were ~0.8 for 10µM ATP and 100µM ATP plates.

3.E. Screening

The following is a suggested protocol; the actual volumes used can be easily adjusted as needed. The main consideration is that the volume of Kinase-Glo® Reagent used should equal the volume of the kinase reaction. The volumes provided in this protocol are intended for a 384-well plate.

1. Add 1µl of compound to each well, except for 16 control wells that should receive 1µl of solvent only.
2. To 8 of the control wells, add 4µl of reaction mixture containing 2.5X the optimal concentration of kinase without kinase substrate in 1X kinase reaction buffer.
3. To all remaining wells, add 4µl of reaction mixture containing 2.5X the optimal concentration of kinase and kinase substrate in 1X kinase reaction buffer.
4. To all wells, add 5µl of reaction mixture containing 2X the desired concentration of ATP in 1X reaction buffer.
5. Mix the plate and incubate for the optimal amount of time.

   We strongly advise optimizing the kinase reaction conditions so that the reaction can be run at room temperature to avoid formation of temperature gradients.

   Note: If the kinase reaction was not run at room temperature, incubate the plate at room temperature BEFORE adding the Kinase-Glo® Reagent.
6. To each well, add 10µl of the appropriate Kinase-Glo® Reagent. Choice of reagent will depend on the ATP concentration in the assay. See Section 3.C.
7. Mix the plate, and incubate for 10 minutes at room temperature. Because of the long half-life of the Kinase-Glo® signal, the plates may be left longer at room temperature before reading, if desired.
8. Record luminescence.

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

3.F. Determining IC_{50} Values of Kinase Inhibitors

The volumes provided in this protocol are intended for a 384-well plate. Other volumes may be used, provided the 1:1 ratio of kinase reaction volume to Kinase-Glo® Reagent is maintained.

1. To each well in columns 2 though 12, add 5µl of reaction mixture containing 1X kinase reaction buffer and 2X the optimal concentration of kinase and kinase substrate.
2. To the wells in column 1, add 10µl of the compound being characterized diluted in the above reaction mixture.
3. Make twofold serial dilutions across the plate. Discard the 5µl removed from the last column.
4. To all wells, add 5µl of reaction mixture containing 1X kinase reaction buffer and 2X the optimal concentration of ATP.
5. Mix the plate and incubate for the optimal amount of time.

   We strongly advise optimizing the kinase reaction conditions so that the reaction can be run at room temperature to avoid formation of temperature gradients.

   Note: If the kinase reaction was not run at room temperature, incubate the plate at room temperature BEFORE adding the Kinase-Glo® Reagent.
6. Add 10µl of the appropriate Kinase-Glo® Reagent to all wells. Choice of reagent will depend on ATP concentration. See Section 3.C.
7. Mix the plate. To stabilize the luminescent signal, equilibrate the plate to room temperature for 10 minutes. The long half-life of the Kinase-Glo® signal allows plates to be left longer at room temperature, if desired.
8. Record luminescence.

   The data in Figures 7 and 8 show titration of an ATP-competitive (H-89) and ATP-noncompetitive (PKI) inhibitor of PKA at different ATP concentrations. Similar IC_{50} were obtained for both inhibitors as reported in the literature at 10µM ATP (5–7). The IC_{50} for H-89 increased significantly with increasing ATP concentrations, while minimal changes in IC_{50} were observed for PKI, confirming the competitive nature of inhibition by H-89 (6,7) and noncompetitive inhibition by PKI (5).

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

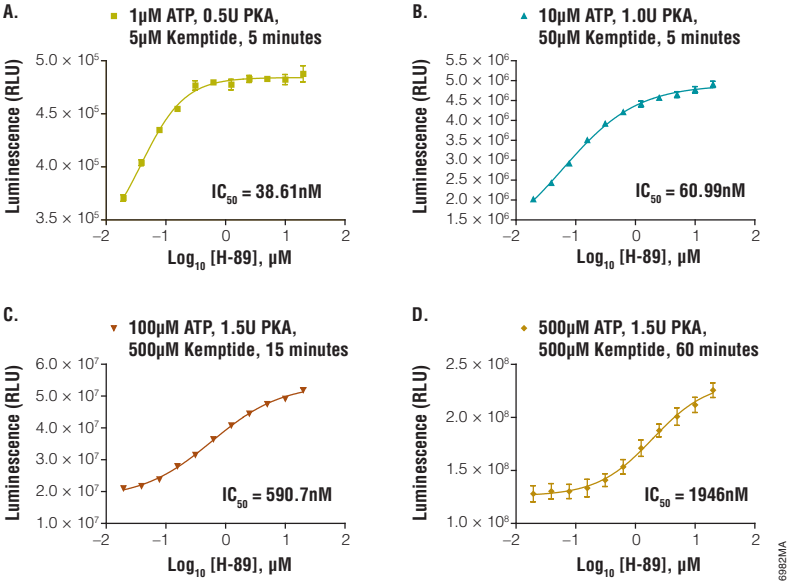

Figure 7. Determining IC_{50} for ATP-competitive inhibitors. PKA inhibitor titrations were performed in solid white, flat-bottom 96-well plates in a total volume of 50µl as described in Figure 4 using indicated amounts of PKA and varying the concentrations of H-89 as shown in the figure. Reactions were carried out at 1µM ATP, 5µM Kemptide substrate and 0.5 units of PKA (Kinase-Glo® Assay, **Panel A**); 10µM ATP, 50µM Kemptide and 1 unit of PKA (Kinase-Glo® Assay, **Panel B**); 100µM ATP, 500µM Kemptide and 1.5 unit of PKA (Kinase-Glo® Plus Assay, **Panel C**); or 500µM ATP, 500µM Kemptide and 1.5 unit of PKA (Kinase-Glo® Max Assay, **Panel D**). Reactions were run at room temperature for 5 minutes (1 and 10µM ATP), 15 minutes for 100µM ATP and 60 minutes for 500µM ATP. Data points are the average of two determinations, and error bars are ± S.D. IC_{50} results determined using the Kinase-Glo® Max Assay are increasing for H-89 with increase in ATP concentration . These compare favorably to the IC_{50} values reported for these compounds in the literature [0.04µM for H-89 at 10µM ATP (6,7)]. Curve fitting was performed using GraphPad Prism® sigmoidal dose-response (variable slope) software.

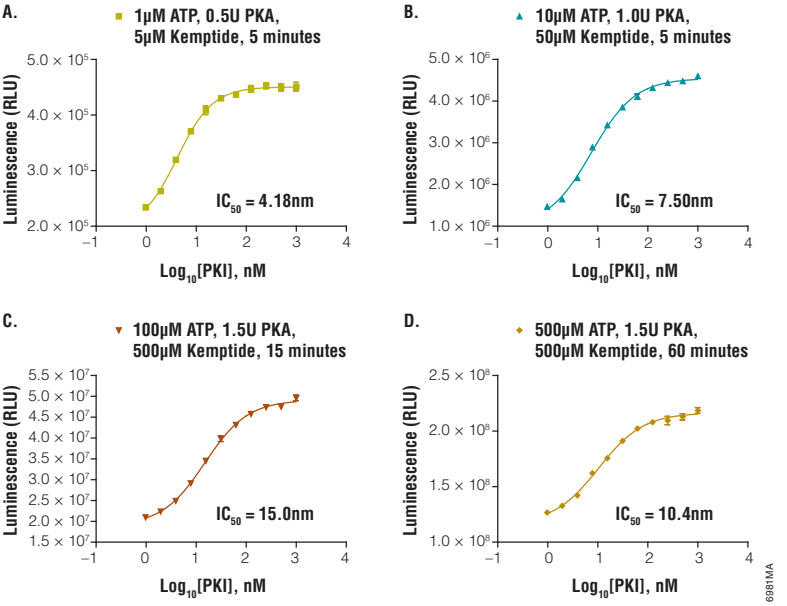

Figure 8. Determining IC_{50} for ATP-noncompetitive inhibitors. PKA inhibitor titrations were performed in solid white, flat-bottom 96-well plates in a total volume of 50µl as described in Figure 4 using indicated amounts of PKA and varying the concentrations of PKI as shown in the figure. Reactions were carried out at 1µM ATP, 5µM Kemptide substrate and 0.5 units of PKA (Kinase-Glo® Assay, **Panel A**); 10µM ATP, 50µM Kemptide and 1 unit of PKA (Kinase-Glo® Assay, **Panel B**); 100µM ATP, 500µM Kemptide and 1.5 unit of PKA (Kinase-Glo® Plus Assay, **Panel C**); or 500µM ATP, 500µM Kemptide and 1.5 unit of PKA (Kinase-Glo® Max Assay, **Panel D**). Reactions were run at room temperature for 5 minutes (1 and 10µM ATP), 15 minutes for 100µM ATP and 60 minutes for 500µM ATP. Data points are the average of two determinations, and error bars are ± S.D. IC_{50} results determined using the Kinase-Glo® Max Assay show with minimal change for PKI with increasing ATP concentrations. These compare favorably to the IC_{50} values reported for these compounds in the literature [3nM for PKI (5)]. Curve fitting was performed using GraphPad Prism® sigmoidal dose-response (variable slope) software.

4. General Considerations

Temperature: The intensity and rate of decay of the luminescent signal from the Kinase-Glo® 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 the 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 Kinase-Glo® Reagent. Insufficient equilibration may result in a temperature gradient between the wells in the center and on the edge of the plates and therefore variability across the plate.

Solvents: The chemical environment of the luciferase reaction will affect the enzyme reaction rate and thus luminescence intensity. Some solvents used to resuspend the various chemical compounds tested may interfere with the luciferase reaction and thus the light output from the assay. Interference with the luciferase reaction can be determined by assaying a parallel set of control wells without kinase or kinase substrate. Dimethylsulfoxide (DMSO), commonly used as a vehicle to solubilize organic chemicals, has been tested at final concentrations up to 2% in the assay and has a minimal effect on light output.

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.

Inhibition of Luciferase: Compounds that only inhibit the kinase will result in higher luminescence compared to solvent-only controls and are easily distinguishable from compounds that only inhibit luciferase activity, which decrease luminescence of a no-kinase control. Compounds that inhibit both the luciferase and kinase, however, might increase, decrease, or have no effect on luminescence depending on the level of inhibition directed toward the kinase and luciferase. Compounds that inhibit luciferase often result in false negatives. However, the unique combination of Ultra-Glo™ Recombinant Luciferase and the proprietary buffer significantly reduces the number of false-negative results (2–4).

5. References

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6. Related Products

Non-Radioactive Protein Kinase Assay Systems

Product	Size	Cat.#
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

6. Related Products (continued)

SAM²® Biotin Capture Membranes

Product	Size	Cat.#
SAM²® Biotin Capture Membrane	96-sample	V2861
	7.6 × 10.9cm	V7861
SAM²® 96 Biotin Capture Plate	96-well plate	V7541
	5 × 96-well plate	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

7. Summary of Changes

This 2/15 update was made to remove expired license statements.

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

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