A Veritas™ Microplate Luminometer Method for

# Promega Kinase-Glo™ Luminescent Kinase Assay

## 1. INTRODUCTION

The Turner BioSystems Veritas™ Microplate Luminometer in combination with Promega's Kinase-Glo™ Luminescent Kinase Assay kit provides a convenient, rapid, and sensitive procedure for quantifying the amount of ATP remaining in solution after a kinase reaction. Kinases constitute a large class of enzymes that phosphorylate specific amino acids according to their original recognition motif. Consequently, kinases play a key role in cell signal transduction and represent exciting new targets for drug research. Scientific developments validate the potential utility of kinases, including serine/threonine, cAMP-dependent, casein, and MAP kinases as specific and important pharmaceutical leads.

Promega's Kinase-Glo™ Assay kit is a homogenous method for virtually any kinase and substrate combination using a non-radioactive luciferase assay. The kinase substrate can be a peptide, protein, or lipid. Luciferase enzyme is regularly used to study a wide range of biological events in cultured cells. Endogenous luciferase activity is absent in mammalian cells, and luciferase based-assays are swift, dependable and easy to perform. The UltraGlow™ Luciferase used in the Kinase-Glo™ Luminescent Kinase Assay kit generates a stable, glow-type signal that has a half-life of greater than four hours. This extended signal allows for batch-mode processing of multiple plates.

Luciferase enzyme requires ATP in order to generate light, and kinases utilize ATP for phosphorylation events. Once the kinase reaction is complete, an equal volume of Kinase-Glo™ is added and luminescence is measured.

Light signal is proportional to the amount of ATP present and is inversely proportional to the amount of kinase activity (Figure 1). It is strongly recommended to optimize assay conditions for superior performance.

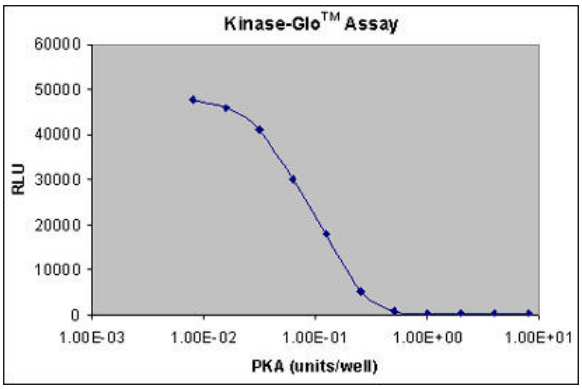


***Figure 1**. Luminescent signal is inversely correlated with kinase activity. Serial two-fold dilutions of PKA in 50 µL kinase reaction buffer (40 mM Tris [pH 7.5], 20 mM MgAc, 0.1% BSA) containing 5 µM Kemptide substrate (Promega P/N V5601) and 1 µM ATP. Kinase-Glo™ Reagent was added following a kinase reaction incubation period of 30 minutes at room temperature. Luminescence was measured with a 1 second/well integration time on the Veritas™ Microplate Luminometer.*

The Veritas™ Microplate Luminometer conveniently offers high sensitivity and a broad dynamic range. The Kinase-Glo™ Luminescent Kinase Assay System has been developed specifically to maximize the sensitivity of the assay reagent and still provide a long-lasting luminescent signal. The Kinase-Glo™ Reagent is compatible with most commonly used organic solvents including DMSO. The Veritas™ Microplate Luminometer can detect as little as $2.01X10^{-15}$ moles ATP using Kinase-Glo™ Substrate. Measurements are linear from 1 pg to 1 ng ATP or three orders of magnitude (Figure 2). All tests were performed using ATP standard (P/N# FF2000).

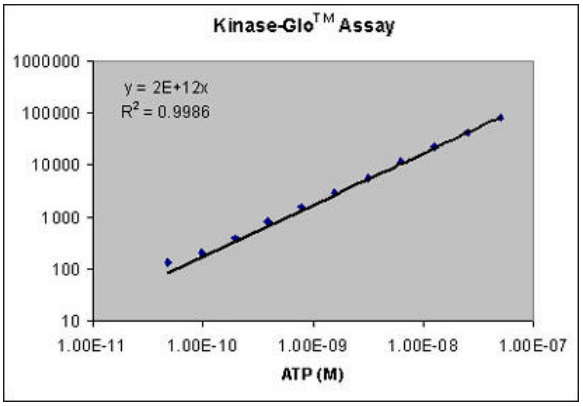


***Figure 2**. Luminescent signal as a function of ATP concentration. Serial two-fold dilutions of ATP in 50 µL kinase reaction buffer (40 mM Tris [pH 7.5], 20 mM MgAc, 0.1% BSA). Following the addition of Kinase-Glo™ Reagent, the plate was incubated at room temperature for 10 minutes before measurement with the Veritas™ Microplate Luminometer.*

## 2. MATERIALS REQUIRED

- Veritas™ Microplate Luminometer (P/N: 9100-000)
- 96-well plates, white (E&K Scientific EK-25075)
- Promega Kinase-Glo™ Luminescent Kinase Assay kit (Promega Catalog#'s V6711, V6712, V6713, V6714)
- p200 pipette and pipette tips

## 3. EXPERIMENT PROTOCOL

### 3.1 Reagent Preparation

**Kinase-Glo™ Substrate:** Use as supplied. Store at -20°C.

**Kinase-Glo™ Buffer:** Use as supplied. Store at -20°C.

**Kinase-Glo™ Reagent:** Thaw Kinase-Glo™ Buffer and equilibrate to room temperature. For convenience, buffer may be thawed and stored at room temperature for 48 hours prior to use. Equilibrate the lyophilized Kinase-Glo™ substrate to room temperature. Transfer entire contents of one bottle of Kinase-Glo™ Buffer to one bottle of Kinase-Glo™ Substrate. Mix gently by inversion until the substrate is thoroughly dissolved, approximately one minute. Use reconstituted reagent immediately or store in small aliquots at -20°C.

**Note:** The temperature of the Kinase-Glo™ Reagent should be held constant at room temperature while quantifying luminescence since luciferase activity is temperature dependent. Reagent stored frozen after reconstitution must be thawed below 25°C to ensure reagent performance. Mix well after thawing. The simplest method for thawing is placing the reagent in a water bath at room temperature.

### 3.2 Instrument Setup

3.2.1 Double click on the Veritas icon to start the software.

3.2.2 Select "Run Promega Protocol" from the "Welcome to Veritas" dialog box.

3.3.3 Select "KinaseGlo" from the list of Promega protocols.

3.3.4 Enter your information into the "Experiment", "Operator", "Plate No.", and "Notes" fields in the "Main Dialog Box".

3.3.5 Select "Options" from the "Main Dialog Box" to select the wells or to modify number of runs. Once modified, click "Apply Changes" and return to the "Main Dialog Box".

## 4. OPTIMIZATION

It is strongly recommended to optimize the kinase reaction with respect to the amount of ATP and kinase substrate. For 96-well plates, we recommend 50 µL Kinase reaction and 50 µL Kinase-Glo™ Reagent for a total of 100 µL. Other volumes may be used, provided the 1:1 ratio of kinase reaction to the Kinase-Glo™ Reagent is maintained.

### 4.1 Determining Optimal ATP Concentration

4.1.1 Make two-fold serial dilution of ATP across the plate using as much kinase as practical and excess kinase substrate. As a control, make the same ATP dilutions without kinase substrate or kinase. Allow the kinase reaction to consume as much ATP as possible. With less active kinases, this may take several hours.

4.1.2. Add an equal volume of Kinase-Glo™ Reagent.

4.1.3. Mix and incubate at room temperature for 10 minutes to allow luminescent signal to stabilize.

4.1.4. Insert plate into the Veritas™ Microplate Luminometer and click on "Start" to begin assay. The optimal ATP concentration will result in the largest change in luminescence in the completed reaction when compared to the wells that are missing kinase and kinase substrate.

**4.2 Determining Optimal Kinase Substrate Concentration**

4.2.1 Make two-fold serial dilution of kinase substrate across the plate using as much kinase as practical and the optimal amount of ATP as determined in 4.1. As a control, do the same titration without kinase. Allow the kinase to phosphorylate as much substrate as possible. With less active kinases, this may take several hours.

4.2.2 Add an equal volume of Kinase-Glo™ Reagent.

4.2.3 Mix and incubate at room temperature for 10 minutes to allow luminescent signal to stabilize.

4.2.4 Insert plate into the Veritas™ Microplate Luminometer and click on "Start" to begin assay. The optimal substrate concentration will result in the largest change in luminescence in the completed reaction when compared to the wells that do not contain kinase.

**4.3 Determining Optimal Kinase Concentration**

4.3.1 Make two-fold serial dilution of kinase across the plate using the optimal amount of ATP as determined in 4.1. and the optimal amount of kinase substrate determined in 4.2. Allow the kinase to consume as much ATP and phosphorylate as much substrate as possible. With less active kinases, this may take several hours.

4.3.2 Add an equal volume of Kinase-Glo™ Reagent.

4.3.3 Mix and incubate at room temperature for 10 minutes to allow luminescent signal to stabilize.

4.3.4 Insert plate into the Veritas™ Microplate Luminometer and click "Start" to begin assay. The optimal kinase concentration should be an amount that results in luminescence in the linear range of the kinase titration curve.

**5. SAMPLE ANALYSIS**

5.1 Mix optimal ATP, kinase, and kinase substrate concentrations with compounds to be screened and incubate for an appropriate amount of time to allow kinase to consume ATP. Add an equal volume of Kinase-Glo™ Reagent. Mix and incubate at room temperature for 10 minutes to allow luminescent signal to stabilize.

5.2 Insert plate into the Veritas™ Microplate Luminometer and click on "Start" to begin assay. RLU values measured by the Veritas™ Microplate Luminometer will appear in the Excel spreadsheet after each row of the selected wells have been read. If you encounter an error message, refer to the troubleshooting guide for more information.

**NOTE:** Please make sure to remove your plate once the measurements are complete.

**6. ABOUT PROMEGA CORPORATION**

Kinase-Glo is a trademark of Promega Corporation. Orders for Promega's products may be placed by:

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Fax: (800) 356-1970
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Mailing Address:

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Madison, WI 53711
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## 7. ABOUT TURNER BIOSYSTEMS

Veritas is a trademark of Turner BioSystems, Inc. Orders for Turner BioSystems' products may be placed by:

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**CAUTION:** The lyophilized Kinase-Glo™ Substrate contains dithiothreitol (DTT) and is therefore classified as hazardous. The reconstituted reagent is not known to present any hazards as the concentration of DTT is less than 1%. However, we recommend the use of gloves, lab coats and eye protection when working with these or any chemical reagents. Promega and Turner BioSystems assume no liability for damage resulting from handling or contact with these products.