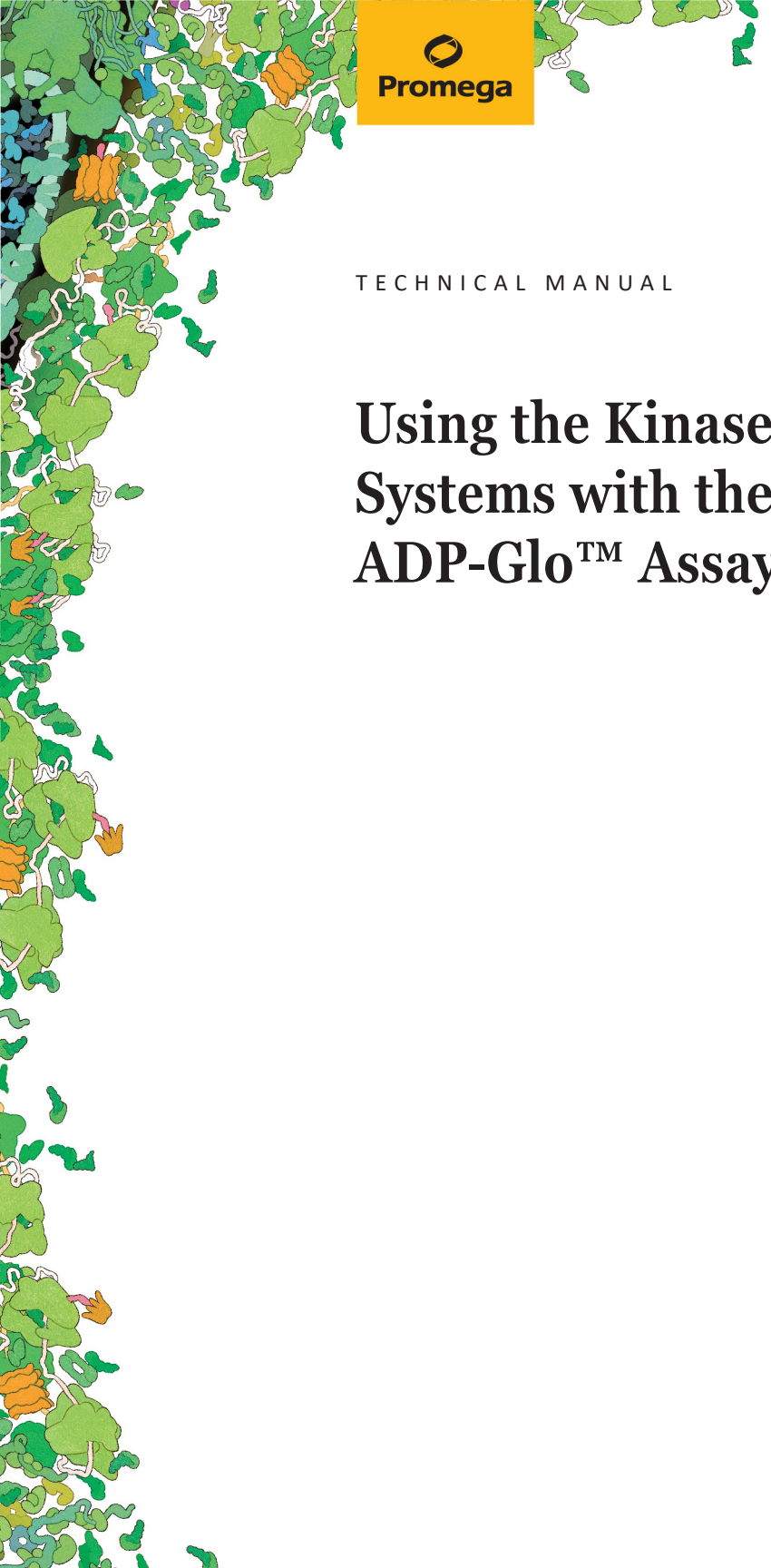

TECHNICAL MANUAL

Using the Kinase Enzyme Systems with the ADP-Glo™ Assay

Revised 4/22
TM553

Using the Kinase Enzyme Systems with the ADP-Glo™ Assay

All technical literature is available at: www.promega.com/protocols/
Visit the web site to verify that you are using the most current version of this Technical Manual.
E-mail Promega Technical Services if you have questions on use of this system: techserv@promega.com

1. Description

The Kinase Enzyme Systems include a recombinant kinase enzyme, a substrate appropriate for the enzyme, a reaction buffer and supplemental reagents as needed. Together, the ADP-Glo™ Assays + Kinase Enzyme Systems provide a convenient method for profiling the effect of chemical compounds on kinase activity.

The ADP-Glo™ Kinase Assays (Cat.# V6930, V9101, V9102) are luminescent ADP detection assays that provide universal, homogeneous, high-throughput screening methods to measure kinase activity by quantifying the amount of ADP produced during a kinase reaction. The luminescent signal positively correlates with kinase activity. The assays are well suited for measuring the effects that chemical compounds have on the activity of a broad range of purified kinases, making them ideal for both primary screening and kinase selectivity profiling. The ADP-Glo™ Kinase Assays 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™ Max Assays (Cat.# V7001, V7002) can be used when higher concentrations (up to 5mM) are required.

After the kinase reaction, the detection assay is performed in two steps. First, an equal volume of ADP-Glo™ Reagent is added to terminate the kinase reaction and deplete the remaining ATP. Second, the Detection Reagent is added to simultaneously convert ADP to ATP and allow the newly synthesized ATP to be measured using a luciferase/luciferin reaction (Figure 1). The light generated is measured using a luminometer. Luminescence can be correlated to ADP concentrations by using an ATP-to-ADP conversion curve (Figure 2).

1. Description (continued)

The ADP-Glo™ Kinase Assays have a high dynamic range and produce a strong signal at low ATP-to-ADP conversion, making them well suited for screening low-activity kinases such as growth factor receptor tyrosine kinases. The assays produce minimal false hits and Z´ values of greater than 0.7 (1).

The ADP-Glo™ Assays are highly sensitive as demonstrated by the high signal-to-background (SB) ratios. Sensitivity is measured by SB at low concentrations of enzyme or ADP production. The robustness of the ADP-Glo™ Kinase Assay and suitability for high-throughput applications is evidenced by high Z´-factor values reported in previous studies (1).

The ADP-Glo™ Kinase Assays are as sensitive as radioactivity-based methods and more sensitive than fluorescence-based technologies (1–3). In order to lower the background and further improve the sensitivity of the assay, we increased the purity of our ATP to have less ADP contamination. To assess the importance of ATP purity on ADP-Glo™ assay sensitivity, we compared the signal-to-background ratios generated in an ADP-Glo™ assay using Promega Ultra Pure ATP and ATP from other suppliers. The Promega Ultra Pure ATP outperforms ATP from other sources by greatly improving ADP-Glo™ Assay sensitivity with SB ratios that are 2–3 times higher than those produced using other commercial preparations (4).

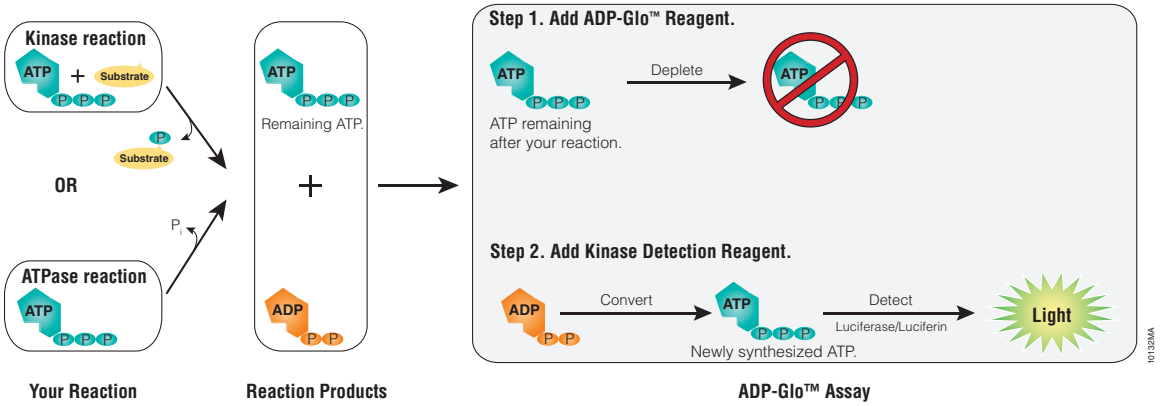

Figure 1. The ADP-Glo™ Assay principle. The detection assay is composed of two steps. After the kinase or ATPase reaction, the first step is performed by adding ADP-Glo™ Reagent, which terminates the kinase reaction and depletes any remaining ATP (40-minute incubation time). Addition of a second reagent converts ADP to ATP and generates light from the newly synthesized ATP using a luciferase/luciferin reaction (incubation is 30–60 minutes depending on the ATP concentration used in the kinase reaction). The light generated is proportional to ADP present and, consequently, kinase or ATPase activity. The assay is performed at room temperature and is compatible with automation.

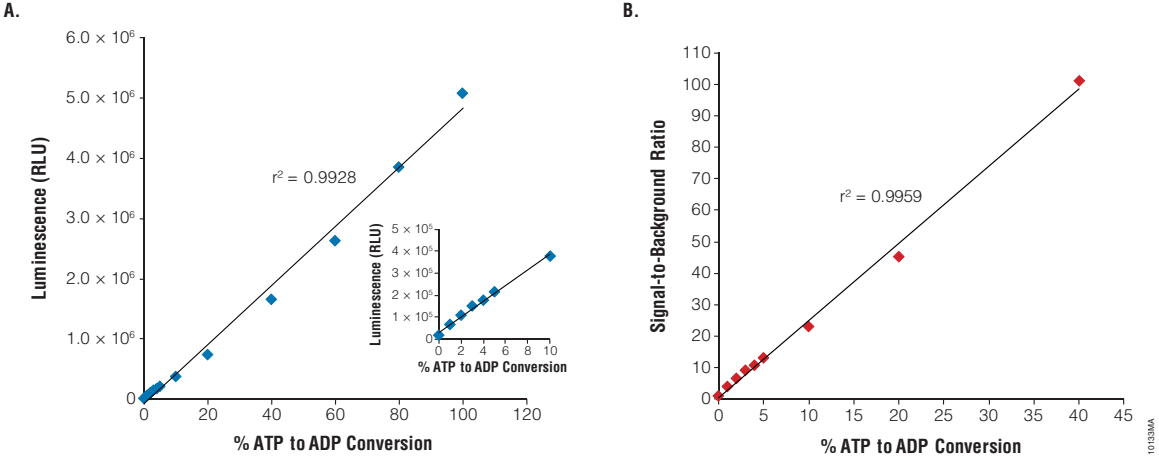

Figure 2. Linearity and sensitivity of the ADP-Glo™ Kinase Assay. The 1mM ATP-to-ADP percent conversion curve (standard curve) was prepared in 1X reaction buffer A (40mM Tris [pH 7.5], 20mM $MgCl_2$ and 0.1mg/ml BSA) without kinase present as described in *ADP-Glo™ Kinase Assay Technical Manual* #TM313 (5). The standards were created by combining the appropriate volumes of ATP and ADP 1mM stock solutions. A 5µl aliquot of each ATP + ADP standard was transferred to a white, opaque 384-well plate. The ADP-Glo™ Kinase Assay was performed by adding 5µl of ADP-Glo™ Reagent and 10µl of Kinase Detection Reagent at room temperature to each well. ADP-Glo™ assay reagents were dispensed in 384-well plates using Multidrop® Combi nL liquid dispenser (Thermo Fisher Scientific). Luminescence values represent the mean of four replicates (RLU = relative light units). **Panel A.** Linearity of the assay up to 1mM ADP. **Panel B.** Sensitivity of the assay is shown as signal-to-background ratios (SB) over a wide range of percent ATP-to-ADP conversion.

2. Protocol for Performing the ADP-Glo™ Assay

Note: Store Kinase Enzyme System components at –70°C. For optimal storage, aliquot target into smaller quantities after centrifugation and store at recommended temperature. For most favorable performance, avoid repeated handling and multiple freeze-thaw cycles. Kinase Enzyme System components are stable for 1 year from the shipment date when stored at –70°C.

Materials to Be Supplied by the User

- ADP-Glo™ Kinase Assay (Cat.# V6930, V9101, V9102, V9103, V9104) or ADP-Glo™ Max Assay (Cat.# V7001, V7002) and related protocols (*ADP-Glo™ Kinase Assay Technical Manual* #TM313 or *ADP-Glo™ Max Assay Technical Manual*, #TM343)
- solid white multiwell plates (do not use black plates)
- multichannel pipette or automated pipetting station
- plate shaker
- luminometer capable of reading multiwell plates

2.A. Kinase Detection Reagent Preparation

1. Thaw the Kinase Detection Buffer at room temperature and look for any precipitate.
2. If a precipitate is present, incubate the Kinase Detection Buffer at 37°C with constant swirling for 15 minutes.
3. Equilibrate the Kinase Detection Buffer and the Kinase Detection Substrate to room temperature before use.
4. Transfer the entire volume of Kinase Detection Buffer into the amber bottle containing the Kinase Detection Substrate to reconstitute the lyophilized substrate. This forms the Kinase Detection Reagent.
5. 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.
6. The Kinase Detection Reagent should be used immediately or dispensed into aliquots and stored at –20°C. The Kinase Detection Reagent is stable for up to 10 freeze-thaw cycles.

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

To estimate the amount of ADP produced in the 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 or ATPase reaction. These standard curves represent the amounts of ATP and ADP available in a reaction at the specified conversion percentage (Table 1). The standard samples used to generate an ATP-to-ADP standard are created by combining the appropriate volumes of ATP and ADP stock solutions. For more information on generating standard curves, see the *ADP-Glo™ Kinase Assay Technical Manual* #TM313 (5) or the *ADP-Glo™ Max Assay Technical Manual* #TM343 (6) and Section 3.A.

Table 1. Percent Conversion of ATP to ADP Represented by the Standard Curve.

	Well 1	Well 2	Well 3	Well 4	Well 5	Well 6	Well 7	Well 8	Well 9	Well 10	Well 11	Well 12
%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

2.C. ADP-Glo™ Kinase Assay Protocol

1. Perform a kinase reaction using 1X kinase buffer (see Section 3 for details).
2. Add ADP-Glo™ Reagent to stop the kinase reaction and deplete the unconsumed ATP, leaving only ADP and a very low background of ATP.
3. Incubate at room temperature for 40 minutes.
4. Add 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.
6. Measure the luminescence with a plate-reading luminometer or charge-coupled device (CCD) camera.

This is a general protocol. Please see the appropriate Technical Manuals for specific details and notes. To screen for kinase inhibitors using the ADP-Glo™ Kinase Assay Systems, see *ADP-Glo™ Kinase Assay Technical Manual* #TM313 (5) for the ADP-Glo™ Kinase Assay or *ADP-Glo™ Max Assay Technical Manual* #TM343 (6) for the ADP-Glo™ Max Assay.

3. Sample Protocol for a Kinase Inhibitor (Staurosporine) Dose-Response Curve

A kinase titration will be performed in order to determine the optimal amount of enzyme to use in subsequent inhibitor dose-response curve determination. To estimate the amount of ADP produced in a kinase reaction, create an ADP standard curve, named "ATP-to-ADP Conversion Curve". This curve represents the luminescence (RLU) corresponding to each percent conversion of ATP-to-ADP based on the ATP concentration used in the kinase reaction. 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. Kinase titrations and ATP-to-ADP conversion curves for similar ATP concentrations will be performed in one plate.

The percent ADP produced by each amount of enzyme is calculated using the reference RLUs from the conversion curves. By titrating the kinase, we will determine SB10. The SB10 value is the amount of the kinase needed to generate a percent conversion reflecting the initial rate of the reaction. We usually use a 5 or 10% conversion because it typically generates a signal-to-background ratio greater than tenfold.

Using the SB10 amount of the kinase, perform a kinase inhibitor (staurosporine) dose-response curve to calculate the IC_{50} and check for any ATPase contaminating activity that will not be inhibited.

Reaction Buffers Needed Using 5X Reaction Buffer A

- 5X Reaction Buffer A: 200mM Tris [pH 7.5], 100mM $MgCl_2$ and 0.5mg/ml BSA
- 4X Kinase Buffer: 4X Reaction Buffer A + 200µM DTT + (4X of any cofactors, e.g., $MnCl_2$)
- 4X Kinase Buffer D: 4X Reaction Buffer A + 200µM DTT + 4% DMSO+ (4X of any cofactors, e.g., $MnCl_2$)
- 1X Kinase Buffer made by diluting the 4X Kinase Buffer
- 1X Kinase Buffer D made by diluting 4X Kinase Buffer D
- 1X Kinase Buffer (5% DMSO) made by diluting the 4X Kinase Buffer and adding 5% DMSO

Note: All volumes described here are for duplicate samples. If you need to perform more than two replicates per sample, recalculate the volumes accordingly.

Perform all steps at room temperature (22–25°C).

3.A. Kinase Titration and Determination of SB10

Generate ATP-to-ADP Conversion Curves

1. Prepare 1mM and 10mM ATP and ADP stock solutions by diluting in water.
2. **Prepare 10X Conversion Curve Standards:** Prepare 10X ADP/ATP stock plates in water as described in Table 2 to make 100µl stock solutions of ATP/ADP standards. You will need 1ml of the 10X ATP and 500µl of the 10X ADP.

   Note: If you are working with only one ATP concentration, make only the corresponding 10X stocks.

Table 2. ATP and ADP Stock Solution Preparation.

	Final Concentration Desired	Prepare This 10X Stock	1mM ATP (µl)	Water (µl)
ATP	1µM	10µM	10	990
	5µM	50µM	50	950
	10µM	100µM	100	900
	Final Concentration Desired	**Prepare This 10X Stock**	**10mM ATP (µl)**	**Water (µl)**
	100µM	1.0mM	100	900
	250µM	2.5mM	250	750
	500µM	5mM	500	500
ADP	**Final Concentration Desired**	**Prepare This 10X Stock**	**1mM ADP (µl)**	**Water (µl)**
	1µM	10µM	5	495
	5µM	50µM	25	475
	10µM	100µM	50	450
	Final Concentration Desired	**Prepare This 10X Stock**	**10mM ADP (µl)**	**Water (µl)**
	100µM	1.0mM	50	450
	250µM	2.5mM	125	375
	500µM	5mM	250	250

3. After you have prepared your ATP and ADP stock solutions, create 10X conversion curve stocks by transferring the amounts of each solution as described in Table 3.

Table 3. 10X Conversion Curve Preparative Plate.

	Well 1	Well 2	Well 3	Well 4	Well 5	Well 6	Well 7	Well 8	Well 9	Well 10	Well 11	Well 12
% Conversion	100	80	60	40	20	10	5	4	3	2	1	0
ADP (µl)	100	80	60	40	20	10	5	4	3	2	1	0
ATP (µl)	0	20	40	60	80	90	95	96	97	98	99	100

3.A. Kinase Titration and Determination of SB10 (continued)

Important Note: Use the remaining 100% ATP from your conversion curve plate to run the kinase reaction in order to have similar background levels.

4. **Prepare a *1X* ADP/ATP working dilution plate in 1X kinase reaction buffer:** Mix 105µl of 4X Kinase Buffer D with 273µl of water. Transfer 27µl/well to a 96-well plate, and then transfer 3µl of the 10X ATP/ADP curve to each respective well in the dilution plate. This will result in a final volume of 30µl, sufficient for 4 replicates, including overage.

Prepare the Kinase Titration Components

1. Prepare the kinase titrations at the same ATP concentrations as the ATP-to-ADP conversion curves.
2. **Prepare Substrate Mix:** For each kinase, prepare 200µl of 2.5X ATP/Substrate Mix in a 1.5ml tube. Use Table 4 as a guideline.

   Note: Use ATP from the same 10X ATP that you used for the conversion curve.

Table 4. Preparation of 2.5X ATP/Substrate Mix.

Component	Volume
4X Kinase Buffer D	50µl
100µM ATP (10X)	50µl
Substrate (1mg/ml)*	100µl

*If substrate is MBP, casein or Histone H1, use 50µl substrate and 50µl water.

3. Transfer 14µl of 2.5X ATP/Substrate Mix to odd numbered wells (1,3,5...23) of a 384-well plate in **Row X**. This is your *ATP/Substrate preparative row*.
4. **Prepare Enzyme Dilution:** Add 10µl of 1X Kinase Buffer D to odd numbered wells, starting with well 3 (3,5,7...23) of the 384-well plate in **Row Y**. Do not add buffer to well 1. This is your *Kinase Dilution preparative row*.
5. Prepare a 20µl kinase solution as described in Table 5 (3µl/reaction/well). This will result in a starting concentration of 200ng kinase/3µl.

Table 5. Preparation of Kinase Solution.

Component	Volume
Water	1.67µl
4X Kinase Buffer D	5µl
Kinase (100ng/µl)	13.33µl

6. Add 20µl of Kinase Solution to well Y1 of the *Kinase Dilution preparative row*. From there, prepare a 1:1 serial dilution of the kinase as shown in Figure 3. Mix well after each dilution by pipetting before transferring 10µl to the next well. Do not continue the serial dilution after well 21.
Note: Do not create bubbles while preparing the dilution series.

Well #	Kinase	Starting Volume of Each Well	Volume to Transfer
1	200ng	20µl	10µl
3	100ng	10µl	10µl
5	50ng	10µl	10µl
7	25ng	10µl	10µl
9	12.5ng	10µl	10µl
11	6.25ng	10µl	10µl
13	3.12ng	10µl	10µl
15	1.56ng	10µl	10µl
17	0.78ng	10µl	10µl
19	0.39ng	10µl	10µl
21	0.1953ng	10µl	0µl
23	0	10µl	No transfer, buffer only

Figure 3. Performing serial 1:1 dilutions of kinase.

7. **Kinase Reaction and Conversion Curve Experiment:** Transfer 5µl of the diluted ATP-ADP series in replicates from your *1X ADP/ATP working dilution plate* into the wells of your 384-well assay plate that are designated for the conversion curve.
8. Transfer 3µl of kinase samples in duplicates from the wells of the *kinase titration preparative, Row Y* to the wells of the assay plate designated for the kinase reactions.
9. Transfer 2µl of the corresponding 2.5X ATP/Substrate Mix from the wells of the *ATP/Substrate preparative Row X* to the same assay rows where the kinase dilutions are.
10. Centrifuge the plate. Mix with a plate shaker for 2 minutes. Incubate the reaction at room temperature for 60 minutes or the desired time.
11. **ADP detection with ADP-Glo™ Kinase Assay:** After the kinase reaction incubation is complete, add 5µl of ADP-Glo™ Reagent to all assay plate wells. Mix for 2 minutes and incubate at room temperature for 40 minutes.
12. Add 10µl of kinase detection reagent to all wells in your assay plate. Mix for 2 minutes and incubate at room temperature for 30–60 minutes.
13. Measure the luminescence (integration time 0.5 second).
14. Calculate the *SB10 value* (ng or nM). *SB10* is the amount needed to generate a signal-to-background ratio of 10 (usually this kinase amount generates a percent conversion of 5 to 10%.).

3.B. Staurosporine Inhibitor Dose Response Curve

1. **Prepare inhibitor titration components:** Add 50µl of 1X Kinase Buffer (with 5% DMSO) to wells A2–B12 of a 96-well plate. These are *inhibitor titration preparative rows*.

   Note: Do not add buffer to well A1.

2. Prepare 100µl of 50µM staurosporine solution (contains 5% DMSO) as described in Table 6 (final 1µl/reaction/well). This will result in 10µM staurosporine (1% DMSO) starting concentration in the assay.

Table 6. Staurosporine Solution Preparation.

Component	Volume
Water	70µl
4X Kinase Buffer	25µl
Staurosporine in DMSO (1mM)	5µl

3. Add 100µl of staurosporine solution to well A1 of the *inhibitor titration preparative rows*. Prepare a 1:1 serial dilution of the inhibitor as shown in Figure 4. Mix well after each dilution by pipetting before transferring into the next well.

   Note: Do not create bubbles while preparing the dilution series.

Well #	Final Inhibitor Concentration	Starting Volume of Each Well	Volume to Transfer
A1	10,000nM	100µl	50µl
A2	5,000nM	50µl	50µl
A3	2,500nM	50µl	50µl
A4	1,250nM	50µl	50µl
A5	625nM	50µl	50µl
A6	312.5nM	50µl	50µl
A7	156.3nM	50µl	50µl
A8	78.1nM	50µl	50µl
A9	39.1nM	50µl	50µl
A10	19.5nM	50µl	50µl
A11	9.8nM	50µl	50µl (transfer to B1)
A12	0	50µl	Buffer Only
B1	4.88nM	50µl	50µl
B2	2.44nM	50µl	50µl
B3	1.22nM	50µl	50µl
B4	0.61nM	50µl	50µl
B5	0.31nM	50µl	50µl
B6	0.15nM	50µl	50µl
B7	0.08nM	50µl	50µl
B8	0.04nM	50µl	50µl
B9	0.02nM	50µl	50µl
B10	0.01nM	50µl	0µl
B11	0	50µl	Buffer Only
B12	No enzyme	10µl	Buffer Only

Figure 4. Performing serial 1:1 dilutions of inhibitor.

3.B. Staurosporine Inhibitor Dose Response Curve (continued)

4. **Prepare Reaction Components, 10µM ATP example:** For each kinase, prepare 200µl of 2.5X ATP/Substrate Mix as described in Table 7.
5. Transfer 14µl of 2.5X ATP/Substrate Mix to odd numbered wells (1,3,5...23) of a 384-well plate in **Row X**. This is your *ATP/Substrate preparative row*.
6. Prepare 140µl of kinase solution (excess amount of 70 reactions at 2µl/reaction/well) as described in Table 8. This will result in an SB10 (ng kinase/reaction).

Table 7. Substrate Mix Preparation.

Component	Volume
4X Kinase Buffer	50µl
100µM ATP (10X)	50µl
Substrate (1mg/ml)*	100µl

*If substrate is MBP, casein, or histone H1, use 50µl substrate (1mg/ml) and 50µl water.

Table 8. Kinase Solution Preparation.

Component	Volume
Kinase (100ng/µl)	Xµl = (70 × SB10/100)
4X Kinase Buffer	35µl
Water	Yµl = 105µl−X

7. Add 12µl of the kinase solution to odd numbered wells (1,3,5...21) and 8µl to well Y23 of a 384-well plate as a *kinase preparative row*.
8. **Kinase Reaction Experiment:** Transfer 2µl kinase samples in duplicate from the wells of the *kinase preparative row* to wells A1 through B22 of a 384-well plate.

   Note: Add only 2µl of 1X Kinase Buffer to wells B23–B24 for the no-enzyme control.
9. Transfer 1µl of inhibitor samples in duplicate from the wells of the *inhibitor titration preparative rows* to the corresponding wells of the *assay rows* (well A1 from the 96-well plate to wells A1 and A2 of the 384-well plate, etc.).
10. Mix and incubate at room temperature for 10 minutes.
11. Transfer 2µl of the corresponding 2.5X ATP/Substrate Mix from the wells of the *ATP/Substrate preparative row* to the same *assay rows* where the kinase/inhibitor mixes are present (Well X1 to wells A1 and A2, etc.).
12. Centrifuge the plate. Mix for 2 minutes and then incubate the kinase reaction at room temperature for 60 minutes or for the desired time.

13. **ADP detection with ADP-Glo™ Kinase Assay:** After the kinase reaction incubation, add 5µl of ADP-Glo™ Reagent to all wells in your assay plate. Mix for 2 minutes and incubate the reaction at room temperature for 40 minutes.
14. Add 10µl of Kinase Detection Reagent to all the wells in your assay plate. Mix for 2 minutes and incubate the reaction at room temperature for 30–60 minutes.
15. Measure the luminescence (integration time 0.5 second).
16. **Calculate Percent Enzyme Activity:** First subtract the signal of the negative control (no enzyme and no staurosporine) from each sample's signal. Then use the mean RLU values for the 0% kinase activity (neither compound nor enzyme) and the 100% kinase activity (no compound) to calculate the other percent enzyme activities remaining in the presence of the different dilutions of staurosporine.

4. General Considerations

Luminescence: The ADP-Glo™Kinase Assay generates luminescence as Relative Light Units (RLU). The RLUs generated for the same kinase assay can be very different depending on the instrument used. Therefore, the RLUs generated using any specific kinase enzyme system will be different from those shown in the application note corresponding to that kinase. Application note links can be found on the individual kinase product pages here: **www.promega.com/products/cell-signaling/kinase-assays-and-kinase-biology/**

Signal to Background (SB) ratio: Depending on the kinase studied, the SB value generated by the enzyme depends on its activity. Therefore, each kinase part of our Kinase Enzyme Systems will generate different SB based on its specific activity. Moreover, kinases in general are very sensitive to freeze-thaw cycles and extended time at high temperatures. To prevent decreases in kinase activity, the kinases should be aliquoted and handled quickly once out of the freezer.

Low enzyme activity: Some kinases have inherently low specific activity and a high amount of enzyme is necessary to perform the assay. To increase the overall ADP produced and at the same time use a minimal amount of enzyme, we recommend increasing the incubation time and/or temperature to 30–37°C.

Substrate-independent kinase activity: In vitro, some kinases have intrinsic ATPase activity, which means in the absence of substrate, the kinase phosphorylates water (hydrolysis) and releases ADP. Depending on the kinase used this activity can be detected without substrate. All the kinases available as Kinase Enzyme Systems are tested to show that they have no exogenous ATPase activity by inhibiting the kinase completely with staurosposine or a specific kinase inhibitor. Moreover, if a kinase has ATPase activity, that activity is inhibitable. Additionally, the IC_{50} generated are similar between the no-substrate and with-substrate experiments.

5. References

1. Tai, A.W. *et al.* (2011) A homogeneous and nonisotopic assay for phosphatidylinositol 4-kinases. *Anal. Biochem.* **417**, 97–102.
2. Zegzouti, H. *et al.* (2009) ADP-Glo: A bioluminescent and homogeneous ADP monitoring assay for kinases. *Assay Drug Dev. Technol.* **7(6)**, 560–72.
3. Vidugiriene, J. *et al.* (2009) Evaluating the utility of a bioluminescent ADP-detecting assay for lipid kinases. *Assay Drug Dev. Technol.* **7(6)**, 585–97.
4. Zegzouti, H. *et al.* (2011) Screening and profiling kinase inhibitors with a luminescent ADP detection platform. www.promega.com/resources/pubhub/screening-and-profiling-kinase-inhibitors-with-a-luminescent-adp-detection-platform.
5. *ADP-Glo™ Kinase Assay Technical Manual* #TM313, Promega Corporation.
6. *ADP-Glo™ Max Assay Technical Manual* #TM343, Promega Corporation.

6. Summary of Changes

The following changes were made to the 4/22 revision of this document:

1. In Section 3.B, Step 1, the percentage of DMSO was updated to 5%.
2. Typos were corrected.
2. The cover image was updated.