

TECHNICAL MANUAL

Kinase Selectivity Profiling System

Instructions for Use of Products

V6850, V6851, V6852, V6853, V6854, V6855, V6856, V6857, V6858, V6859, V6910, V6913, V6914, V6915, V6916, V6917, V6918, V6919, V6920, V6921, V6922, V6923, V6924, V6925, V6926, V6927, V6928, V6929, V6931, V6932



Kinase Selectivity Profiling System

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

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1. Description

During the drug discovery process, profiling lead-compound kinase inhibitors against a broad panel of kinases is important to better understand inhibitor activity and obviate any off-target effects, thus promoting drug safety. Moreover, defining specificity of inhibitors towards a specific kinase or group of kinases is useful to the research community in general and to the cell-signaling community in particular (1). Although several technologies exist that attempt to create kinase inhibitor profiles, they are geared towards generating large kinome profiling data (2–5), and most suffer from limitations that prevent their use in routine kinase inhibitor profile creation.

The Kinase Selectivity Profiling Systems include kinase and substrate pairs organized in an easy-to-use 8-tube strip format and optimized for fast and simple kinase-profiling reactions. Kinase Selectivity Profiling Systems offer kinases grouped either in single kinase family strips or as a general panel of kinases representative of the human kinome for a broad kinase profile. Each profiling system contains the reagents needed to complete a profile for several compounds, including kinase reaction buffer, eight kinases in each multiwell strip and eight corresponding substrates and cofactors in another multiwell strip. The kinase stock solutions are standardized in a way that, when kinases are diluted to the final concentration in the kinase reaction, the kinase activity will result in optimal ATP to ADP conversion in 5µl reactions

1. Description (continued)

(384-well plate), with a signal-to-background ratio of greater than or equal to ten when used in conjunction with the ADP-Glo™ Kinase Assay^(a-f) (6). The substrate stock solutions are standardized in a similar fashion and are provided in a second 8-tube strip with the substrates at corresponding positions. An overview of the kinase-profiling protocol is shown in Figure 1. The Kinase Selectivity Profiling Systems are optimized for performance in a 384-well plate and are well suited for high-throughput kinase selectivity profiling and automated setup.

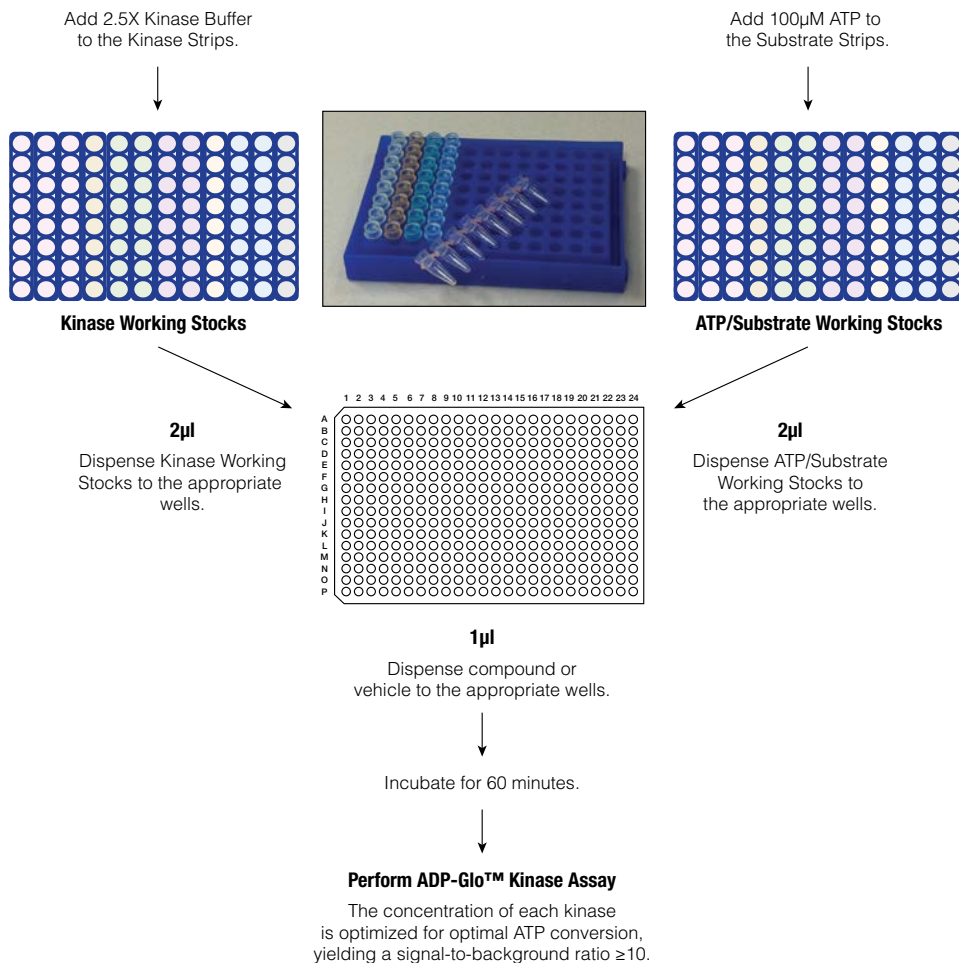


Figure 1. Kinase Selectivity Profiling Systems overview. Kinases are provided at 25X or 50X concentrations in an 8-tube strip, and substrates/cofactors are provided at 3.3X concentrations in a separate 8-tube strip. One-step dilutions directly in these strips produce sufficient Kinase and ATP/Substrate Working Stocks for 25 kinase reactions in a 384-well plate. Kinase reactions are performed using 1µl of compound, 2µl of Kinase Working Stock and 2µl of ATP/Substrate Working Stock. After a 1-hour incubation at room temperature (22–25°C), kinase activity is quantified using the ADP-Glo™ Kinase Assay. The luminescent signal generated by the ADP-Glo™ Kinase Assay is proportional to ADP concentration and is correlated with kinase activity.

The Kinase Selectivity Profiling Systems offer flexible kinase-profiling experimental setup. Each system can be used to generate compound selectivity profiles at one concentration for up to 20 compounds (Figure 2) or as a dose-response curve for two compounds against each kinase in the Kinase Strip (Figure 3). Use the Kinase Selectivity Profiling Systems and the optimized protocols provided here to easily generate selectivity profiles using small or large kinase panels and detect compound promiscuity towards members of a single kinase subfamily or different subfamilies of the kinome.

Single-Dose Profiling

		>60% Activity	20–60% Activity	<20% Activity						
		Kinase	Gefitinib	Dasatinib	Tofacitinib	SB203580	Roscovitine	PF-477736	Tozasertib	Enzastaurin
Kinase Profiling System: TK-3	AXL1	108	92	105	94	102	54	107	101	
	EPHA1	84	1	102	79	97	63	77	100	
	FAK	95	84	118	106	109	86	91	115	
	ITK	102	101	99	94	100	33	24	99	
	JAK3	99	113	0	87	94	36	88	103	
	PYK2	133	134	122	106	134	120	80	116	
	SYK	104	72	84	87	94	34	77	103	
	TRKA	95	82	60	89	87	9	1	89	

Inhibitor Concentration = 1 μ M

Figure 2. Single-dose inhibitor profiles of eight compounds created using the Kinase Selectivity Profiling System: TK-3. Kinase selectivity assays were assembled in a 384-well plate using 1 μ l of the indicated compound (1 μ M final concentration), 2 μ l of each Kinase Working Stock and 2 μ l of the corresponding ATP/Substrate Working Stock. Assays were performed as described in Section 5.B, and kinase activity was quantified using the ADP-Glo™ Kinase Assay. The percent activity of each kinase in the presence of each compound is indicated.

The Kinase Selectivity Profiling Systems are the latest addition to the ADP-Glo™ platform, which is ideal not only for primary and secondary screening but also for profiling compounds in a cost-effective manner using a single platform.

Advantages of Kinase Selectivity Profiling Systems include:

- **Fast and simple reaction assembly:** Two quick dilutions provide working stocks of kinases and substrate/cofactor solutions sufficient for 25 kinase reactions per strip pair.
- **One-time-use design:** Eliminating multiple freeze-thaw cycles ensures optimal kinase activity.
- **Optimized kinase activity for compound profiling:** All kinases were developed for optimal ADP production, with a signal-to-background ratio of greater than or equal to ten when assayed at 10 μ M ATP using the ADP-Glo™ Kinase Assay.
- **Systems provide access to eight kinases at a time:** The Kinase Selectivity Profiling Systems are available in two formats: kinases from single kinase families grouped together for more relevant selectivity profiles or a general panel of kinases representative of the kinome for a broader profile.
- **Flexible kinase compound profiling:** Each pair of Kinase and Substrate/Co-Factor Strips has sufficient material to profile ten compounds at a single dose or create a dose-response curve for one compound against eight kinases in a single experiment. Each system contains two Kinase Strips and two Substrate/Co-Factor Strips; the Kinase Selectivity Profiling System: General Panel contains three Kinase Strips and three Substrate/Co-Factor Strips.

1. Description (continued)

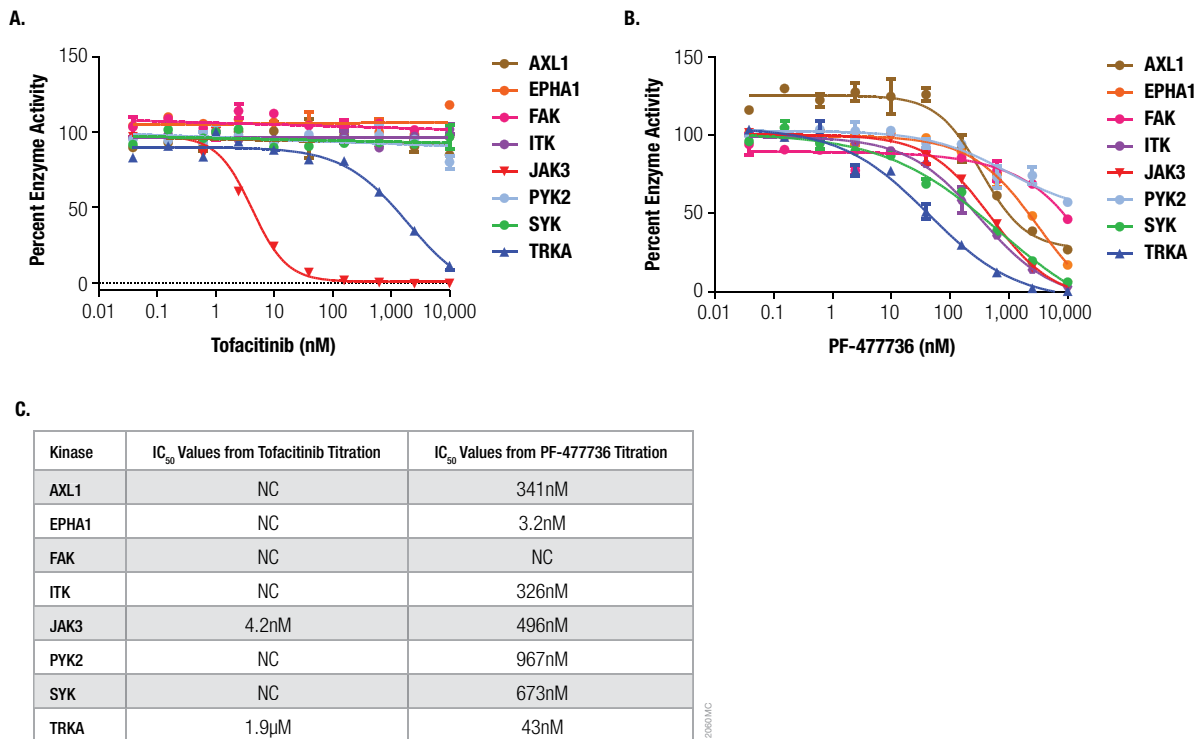


Figure 3. Inhibitor dose-response curves created using the Kinase Selectivity Profiling System: TK-3. The selective JAK3 inhibitor tofacitinib (**Panel A**) and nonselective inhibitor PF-47736 (**Panel B**) were assayed at the indicated concentrations using the Kinase Selectivity Profiling System: TK-3. Kinase assays were assembled using 1 μl of compound, 2 μl of Kinase Working Stock and 2 μl of the corresponding ATP/Substrate Working Stock in a 384-well plate. Assays were performed as described in Section 5.C, and kinase activity was quantified using the ADP-Glo™ Kinase Assay. The percent activity of each kinase in the presence of each compound is indicated. Curves were fit to a sigmoidal dose-response (variable slope) equation, and IC₅₀ values (**Panel C**) were calculated (7) using the Kinase Selectivity Profiling Systems (KPS) Dose-Response SMART Protocol, which can be found at:

www.promega.com/resources/tools/

NC = not calculated.

2. Product Components and Storage Conditions

PRODUCT	SIZE	CAT.#
Kinase Selectivity Profiling System: TK-1 + ADP-Glo™ Assay	8 × 50 reactions	V6851

Kinase Selectivity Profiling System: TK-1 + ADP-Glo™ Assay contains eight kinase/substrate pairs from the tyrosine kinase family (see Figure 4). This system is sufficient for 50 sets of 8 kinase reactions (25 sets of 8 kinase reactions per strip pair) in 384-well plates using 2µl of Kinase Working Stock and 2µl of ATP/Substrate Working Stock. The system also includes sufficient ADP-Glo™ Assay reagents for 400 kinase assays.

Includes:

Cat.# V6850 Kinase Selectivity Profiling System: TK-1, which includes:

- 2 TK-1 Kinase Strip
- 2 TK-1 Substrate/Co-Factor Strip
- 1.5ml 5X Reaction Buffer A
- 25µl DTT, 0.1M

Cat.# V6930 ADP-Glo™ Kinase Assay, which includes:

- 2ml ADP-Glo™ Reagent
- 4ml Kinase Detection Reagent
- 500µl Ultra Pure ATP, 10mM
- 500µl ADP, 10mM

PRODUCT	SIZE	CAT.#
Kinase Selectivity Profiling System: TK-1	8 × 50 reactions	V6850

Kinase Selectivity Profiling System: TK-1 contains eight kinase/substrate pairs from the tyrosine kinase family (see Figure 4). This system is sufficient for 50 sets of 8 kinase reactions (25 sets of 8 kinase reactions per strip pair) in 384-well plates using 2µl of Kinase Working Stock and 2µl of ATP/Substrate Working Stock. Includes:

- 2 TK-1 Kinase Strip
- 2 TK-1 Substrate/Co-Factor Strip
- 1.5ml 5X Reaction Buffer A
- 25µl DTT, 0.1M



2. Product Components and Storage Conditions (continued)

PRODUCT	SIZE	CAT.#
Kinase Selectivity Profiling System: TK-2 + ADP-Glo™ Assay	8 × 50 reactions	V6853

Kinase Selectivity Profiling System: TK-2 + ADP-Glo™ Assay contains eight kinase/substrate pairs from the tyrosine kinase family (see Figure 4). This system is sufficient for 50 sets of 8 kinase reactions (25 sets of 8 kinase reactions per strip pair) in 384-well plates using 2µl of Kinase Working Stock and 2µl of ATP/Substrate Working Stock. The system also includes sufficient ADP-Glo™ Assay reagents for 400 kinase assays. Includes:

Cat.# V6852 Kinase Selectivity Profiling System: TK-2, which includes:

- 2 TK-2 Kinase Strip
- 2 TK-2 Substrate/Co-Factor Strip
- 1.5ml 5X Reaction Buffer A
- 25µl DTT, 0.1M

Cat.# V6930 ADP-Glo™ Kinase Assay, which includes:

- 2ml ADP-Glo™ Reagent
- 4ml Kinase Detection Reagent
- 500µl Ultra Pure ATP, 10mM
- 500µl ADP, 10mM

PRODUCT	SIZE	CAT.#
Kinase Selectivity Profiling System: TK-2	8 × 50 reactions	V6852

Kinase Selectivity Profiling System: TK-2 contains eight kinase/substrate pairs from the tyrosine kinase family (see Figure 4). This system is sufficient for 50 sets of 8 kinase reactions (25 sets of 8 kinase reactions per strip pair) in 384-well plates using 2µl of Kinase Working Stock and 2µl of ATP/Substrate Working Stock. Includes:

- 2 TK-2 Kinase Strip
- 2 TK-2 Substrate/Co-Factor Strip
- 1.5ml 5X Reaction Buffer A
- 25µl DTT, 0.1M

PRODUCT	SIZE	CAT.#
Kinase Selectivity Profiling System: TK-3 + ADP-Glo™ Assay	8 × 50 reactions	V6921

Kinase Selectivity Profiling System: TK-3 + ADP-Glo™ Assay contains eight kinase/substrate pairs from the tyrosine kinase family (see Figure 4). This system is sufficient for 50 sets of 8 kinase reactions (25 sets of 8 kinase reactions per strip pair) in 384-well plates using 2µl of Kinase Working Stock and 2µl of ATP/Substrate Working Stock. The system also includes sufficient ADP-Glo™ Assay reagents for 400 kinase assays. Includes:

Cat.# V6920 Kinase Selectivity Profiling System: TK-3 which includes:

- 2 TK-3 Kinase Strip
- 2 TK-3 Substrate/Co-Factor Strip
- 1.5ml 5X Reaction Buffer A
- 25µl DTT, 0.1M

Cat.# V6930 ADP-Glo™ Kinase Assay, which includes:

- 2ml ADP-Glo™ Reagent
- 4ml Kinase Detection Reagent
- 500µl Ultra Pure ATP, 10mM
- 500µl ADP, 10mM

PRODUCT	SIZE	CAT.#
Kinase Selectivity Profiling System: TK-3	8 × 50 reactions	V6920

Kinase Selectivity Profiling System: TK-3 contains eight kinase/substrate pairs from the tyrosine kinase family (see Figure 4). This system is sufficient for 50 sets of 8 kinase reactions (25 sets of 8 kinase reactions per strip pair) in 384-well plates using 2µl of Kinase Working Stock and 2µl of ATP/Substrate Working Stock. Includes:

- 2 TK-3 Kinase Strip
- 2 TK-3 Substrate/Co-Factor Strip
- 1.5ml 5X Reaction Buffer A
- 25µl DTT, 0.1M

PRODUCT	SIZE	CAT.#
Kinase Selectivity Profiling System: TK-4 + ADP-Glo™ Assay	8 × 50 reactions	V6923

Kinase Selectivity Profiling System: TK-4 + ADP-Glo™ Assay contains eight kinase/substrate pairs from the tyrosine kinase family (see Figure 4). This system is sufficient for 50 sets of 8 kinase reactions (25 sets of 8 kinase reactions per strip pair) in 384-well plates using 2µl of Kinase Working Stock and 2µl of ATP/Substrate Working Stock. The system also includes sufficient ADP-Glo™ Assay reagents for 400 kinase assays. Includes:

Cat.# V6922 Kinase Selectivity Profiling System: TK-4, which includes:

- 2 TK-4 Kinase Strip
- 2 TK-4 Substrate/Co-Factor Strip
- 1.5ml 5X Reaction Buffer A
- 25µl DTT, 0.1M

Cat.# V6930 ADP-Glo™ Kinase Assay, which includes:

- 2ml ADP-Glo™ Reagent
- 4ml Kinase Detection Reagent
- 500µl Ultra Pure ATP, 10mM
- 500µl ADP, 10mM



2. Product Components and Storage Conditions (continued)

PRODUCT	SIZE	CAT.#
Kinase Selectivity Profiling System: TK-4	8 × 50 reactions	V6922

Kinase Selectivity Profiling System: TK-4 contains eight kinase/substrate pairs from the tyrosine kinase family (see Figure 4). This system is sufficient for 50 sets of 8 kinase reactions (25 sets of 8 kinase reactions per strip pair) in 384-well plates using 2 μ l of Kinase Working Stock and 2 μ l of ATP/Substrate Working Stock. Includes:

- 2 TK-4 Kinase Strip
- 2 TK-4 Substrate/Co-Factor Strip
- 1.5ml 5X Reaction Buffer A
- 25 μ l DTT, 0.1M

PRODUCT	SIZE	CAT.#
Kinase Selectivity Profiling System: CMGC-1 + ADP-Glo™ Assay	8 × 50 reactions	V6855

Kinase Selectivity Profiling System: CMGC-1 + ADP-Glo™ Assay contains eight kinase/substrate pairs from the CMGC kinase family (see Figure 4). This system is sufficient for 50 sets of 8 kinase reactions (25 sets of 8 kinase reactions per strip pair) in 384-well plates using 2 μ l of Kinase Working Stock and 2 μ l of ATP/Substrate Working Stock. The system also includes sufficient ADP-Glo™ Assay reagents for 400 kinase assays. Includes:

Cat.# V6854 Kinase Selectivity Profiling System: CMGC-1, which includes:

- 2 CMGC-1 Kinase Strip
- 2 CMGC-1 Substrate/Co-Factor Strip
- 1.5ml 5X Reaction Buffer A
- 25 μ l DTT, 0.1M

Cat.# V6930 ADP-Glo™ Kinase Assay, which includes:

- 2ml ADP-Glo™ Reagent
- 4ml Kinase Detection Reagent
- 500 μ l Ultra Pure ATP, 10mM
- 500 μ l ADP, 10mM

PRODUCT	SIZE	CAT.#
Kinase Selectivity Profiling System: CMGC-1	8 × 50 reactions	V6854

Kinase Selectivity Profiling System: CMGC-1 contains eight kinase/substrate pairs from the CMGC kinase family (see Figure 4). This system is sufficient for 50 sets of 8 kinase reactions (25 sets of 8 kinase reactions per strip pair) in 384-well plates using 2 μ l of Kinase Working Stock and 2 μ l of ATP/Substrate Working Stock. Includes:

- 2 CMGC-1 Kinase Strip
- 2 CMGC-1 Substrate/Co-Factor Strip
- 1.5ml 5X Reaction Buffer A
- 25 μ l DTT, 0.1M

PRODUCT	SIZE	CAT.#
Kinase Selectivity Profiling System: CMGC-2 + ADP-Glo™ Assay	8 × 50 reactions	V6857

Kinase Selectivity Profiling System: CMGC-2 + ADP-Glo™ Assay contains eight kinase/substrate pairs from the CMGC kinase family (see Figure 4). This system is sufficient for 50 sets of 8 kinase reactions (25 sets of 8 kinase reactions per strip pair) in 384-well plates using 2µl of Kinase Working Stock and 2µl of ATP/Substrate Working Stock. The system also includes sufficient ADP-Glo™ Assay reagents for 400 kinase assays. Includes:

Cat.# V6856 Kinase Selectivity Profiling System: CMGC-2, which includes:

- 2 CMGC-2 Kinase Strip
- 2 CMGC-2 Substrate/Co-Factor Strip
- 1.5ml 5X Reaction Buffer A
- 25µl DTT, 0.1M

Cat.# V6930 ADP-Glo™ Kinase Assay, which includes:

- 2ml ADP-Glo™ Reagent
- 4ml Kinase Detection Reagent
- 500µl Ultra Pure ATP, 10mM
- 500µl ADP, 10mM

PRODUCT	SIZE	CAT.#
Kinase Selectivity Profiling System: CMGC-2	8 × 50 reactions	V6856

Kinase Selectivity Profiling System: CMGC-2 contains eight kinase/substrate pairs from the CMGC kinase family (see Figure 4). This system is sufficient for 50 sets of 8 kinase reactions (25 sets of 8 kinase reactions per strip pair) in 384-well plates using 2µl of Kinase Working Stock and 2µl of ATP/Substrate Working Stock. Includes:

- 2 CMGC-2 Kinase Strip
- 2 CMGC-2 Substrate/Co-Factor Strip
- 1.5ml 5X Reaction Buffer A
- 25µl DTT, 0.1M

PRODUCT	SIZE	CAT.#
Kinase Selectivity Profiling System: AGC-1 + ADP-Glo™ Assay	8 × 50 reactions	V6859

Kinase Selectivity Profiling System: AGC-1 + ADP-Glo™ Assay contains eight kinase/substrate pairs from the AGC kinase family (see Figure 4). This system is sufficient for 50 sets of 8 kinase reactions (25 sets of 8 kinase reactions per strip pair) in 384-well plates using 2µl of Kinase Working Stock and 2µl of ATP/Substrate Working Stock. The system also includes sufficient ADP-Glo™ Assay reagents for 400 kinase assays. Includes:

Cat.# V6858 Kinase Selectivity Profiling System: AGC-1, which includes:

- 2 AGC-1 Kinase Strip
- 2 AGC-1 Substrate/Co-Factor Strip
- 1.5ml 5X Reaction Buffer A
- 25µl DTT, 0.1M

Cat.# V6930 ADP-Glo™ Kinase Assay, which includes:

- 2ml ADP-Glo™ Reagent
- 4ml Kinase Detection Reagent
- 500µl Ultra Pure ATP, 10mM
- 500µl ADP, 10mM



2. Product Components and Storage Conditions (continued)

PRODUCT	SIZE	CAT.#
Kinase Selectivity Profiling System: AGC-1	8 × 50 reactions	V6858

Kinase Selectivity Profiling System: AGC-1 contains eight kinase/substrate pairs from the AGC kinase family (see Figure 4). This system is sufficient for 50 sets of 8 kinase reactions (25 sets of 8 kinase reactions per strip pair) in 384-well plates using 2 μ l of Kinase Working Stock and 2 μ l of ATP/Substrate Working Stock. Includes:

- 2 AGC-1 Kinase Strip
- 2 AGC-1 Substrate/Co-Factor Strip
- 1.5ml 5X Reaction Buffer A
- 25 μ l DTT, 0.1M

PRODUCT	SIZE	CAT.#
Kinase Selectivity Profiling System: AGC-2 + ADP-Glo™ Assay	8 × 50 reactions	V6931

Kinase Selectivity Profiling System: AGC-2 + ADP-Glo™ Assay contains eight kinase/substrate pairs from the AGC kinase family (see Figure 4). This system is sufficient for 50 sets of 8 kinase reactions (25 sets of 8 kinase reactions per strip pair) in 384-well plates using 2 μ l of Kinase Working Stock and 2 μ l of ATP/Substrate Working Stock. The system also includes sufficient ADP-Glo™ Assay reagents for 400 kinase assays. Includes:

Cat.# V6910 Kinase Selectivity Profiling System: AGC-2, which includes:

- 2 AGC-2 Kinase Strip
- 2 AGC-2 Substrate/Co-Factor Strip
- 1.5ml 5X Reaction Buffer A
- 25 μ l DTT, 0.1M

Cat.# V6930 ADP-Glo™ Kinase Assay, which includes:

- 2ml ADP-Glo™ Reagent
- 4ml Kinase Detection Reagent
- 500 μ l Ultra Pure ATP, 10mM
- 500 μ l ADP, 10mM

PRODUCT	SIZE	CAT.#
Kinase Selectivity Profiling System: AGC-2	8 × 50 reactions	V6910

Kinase Selectivity Profiling System: AGC-2 contains eight kinase/substrate pairs from the AGC kinase family (see Figure 4). This system is sufficient for 50 sets of 8 kinase reactions (25 sets of 8 kinase reactions per strip pair) in 384-well plates using 2 μ l of Kinase Working Stock and 2 μ l of ATP/Substrate Working Stock. Includes:

- 2 AGC-2 Kinase Strip
- 2 AGC-2 Substrate/Co-Factor Strip
- 1.5ml 5X Reaction Buffer A
- 25 μ l DTT, 0.1M

PRODUCT	SIZE	CAT.#
Kinase Selectivity Profiling System: CAMK-1 + ADP-Glo™ Assay	8 × 50 reactions	V6913

Kinase Selectivity Profiling System: CAMK-1 + ADP-Glo™ Assay contains eight kinase/substrate pairs from the CAMK kinase family (see Figure 4). This system is sufficient for 50 sets of 8 kinase reactions (25 sets of 8 kinase reactions per strip pair) in 384-well plates using 2µl of Kinase Working Stock and 2µl of ATP/Substrate Working Stock. The system also includes sufficient ADP-Glo™ Assay reagents for 400 kinase assays. Includes:

Cat.# V6932 Kinase Selectivity Profiling System: CAMK-1, which includes:

- 2 CAMK-1 Kinase Strip
- 2 CAMK-1 Substrate/Co-Factor Strip
- 1.5ml 5X Reaction Buffer A
- 25µl DTT, 0.1M

Cat.# V6930 ADP-Glo™ Kinase Assay, which includes:

- 2ml ADP-Glo™ Reagent
- 4ml Kinase Detection Reagent
- 500µl Ultra Pure ATP, 10mM
- 500µl ADP, 10mM

PRODUCT	SIZE	CAT.#
Kinase Selectivity Profiling System: CAMK-1	8 × 50 reactions	V6932

Kinase Selectivity Profiling System: CAMK-1 contains eight kinase/substrate pairs from the CAMK kinase family (see Figure 4). This system is sufficient for 50 sets of 8 kinase reactions (25 sets of 8 kinase reactions per strip pair) in 384-well plates using 2µl of Kinase Working Stock and 2µl of ATP/Substrate Working Stock. Includes:

- 2 CAMK-1 Kinase Strip
- 2 CAMK-1 Substrate/Co-Factor Strip
- 1.5ml 5X Reaction Buffer A
- 25µl DTT, 0.1M

PRODUCT	SIZE	CAT.#
Kinase Selectivity Profiling System: CAMK-2 + ADP-Glo™ Assay	8 × 50 reactions	V6925

Kinase Selectivity Profiling System: CAMK-2 + ADP-Glo™ Assay contains eight kinase/substrate pairs from the CAMK kinase family (see Figure 4). This system is sufficient for 50 sets of 8 kinase reactions (25 sets of 8 kinase reactions per strip pair) in 384-well plates using 2µl of Kinase Working Stock and 2µl of ATP/Substrate Working Stock. The system also includes sufficient ADP-Glo™ Assay reagents for 400 kinase assays. Includes:

Cat.# V6924 Kinase Selectivity Profiling System: CAMK-2, which includes:

- 2 CAMK-2 Kinase Strip
- 2 CAMK-2 Substrate/Co-Factor Strip
- 1.5ml 5X Reaction Buffer A
- 25µl DTT, 0.1M

Cat.# V6930 ADP-Glo™ Kinase Assay, which includes:

- 2ml ADP-Glo™ Reagent
- 4ml Kinase Detection Reagent
- 500µl Ultra Pure ATP, 10mM
- 500µl ADP, 10mM



2. Product Components and Storage Conditions (continued)

PRODUCT	SIZE	CAT.#
Kinase Selectivity Profiling System: CAMK-2	8 × 50 reactions	V6924

Kinase Selectivity Profiling System: CAMK-2 contains eight kinase/substrate pairs from the CAMK kinase family (see Figure 4). This system is sufficient for 50 sets of 8 kinase reactions (25 sets of 8 kinase reactions per strip pair) in 384-well plates using 2 μ l of Kinase Working Stock and 2 μ l of ATP/Substrate Working Stock. Includes:

- 2 CAMK-2 Kinase Strip
- 2 CAMK-2 Substrate/Co-Factor Strip
- 1.5ml 5X Reaction Buffer A
- 25 μ l DTT, 0.1M

PRODUCT	SIZE	CAT.#
Kinase Selectivity Profiling System: Other/CK-1 + ADP-Glo™ Assay	8 × 50 reactions	V6919

Kinase Selectivity Profiling System: Other/CK-1 + ADP-Glo™ Assay contains eight kinase/substrate pairs from the Other/CK kinase families (see Figure 4). This system is sufficient for 50 sets of 8 kinase reactions (25 sets of 8 kinase reactions per strip pair) in 384-well plates using 2 μ l of Kinase Working Stock and 2 μ l of ATP/Substrate Working Stock. The system also includes sufficient ADP-Glo™ Assay reagents for 400 kinase assays. Includes:

Cat.# V6918 Kinase Selectivity Profiling System: Other/CK-1, which includes:

- 2 Other/CK-1 Kinase Strip
- 2 Other/CK-1 Substrate/Co-Factor Strip
- 1.5ml 5X Reaction Buffer A
- 25 μ l DTT, 0.1M

Cat.# V6930 ADP-Glo™ Kinase Assay, which includes:

- 2ml ADP-Glo™ Reagent
- 4ml Kinase Detection Reagent
- 500 μ l Ultra Pure ATP, 10mM
- 500 μ l ADP, 10mM

PRODUCT	SIZE	CAT.#
Kinase Selectivity Profiling System: Other/CK-1	8 × 50 reactions	V6918

Kinase Selectivity Profiling System: Other/CK-1 contains eight kinase/substrate pairs from the Other/CK kinase families (see Figure 4). This system is sufficient for 50 sets of 8 kinase reactions (25 sets of 8 kinase reactions per strip pair) in 384-well plates using 2 μ l of Kinase Working Stock and 2 μ l of ATP/Substrate Working Stock. Includes:

- 2 Other/CK-1 Kinase Strip
- 2 Other/CK-1 Substrate/Co-Factor Strip
- 1.5ml 5X Reaction Buffer A
- 25 μ l DTT, 0.1M

PRODUCT	SIZE	CAT.#
Kinase Selectivity Profiling System: Other-2 + ADP-Glo™ Assay	8 × 50 reactions	V6927

Kinase Selectivity Profiling System: Other-2 + ADP-Glo™ Assay contains eight kinase/substrate pairs from the Other kinase family (see Figure 4). This system is sufficient for 50 sets of 8 kinase reactions (25 sets of 8 kinase reactions per strip pair) in 384-well plates using 2µl of Kinase Working Stock and 2µl of ATP/Substrate Working Stock. The system also includes sufficient ADP-Glo™ Assay reagents for 400 kinase assays. Includes:

Cat.# V6926 Kinase Selectivity Profiling System: Other-2, which includes:

- 2 Other-2 Kinase Strip
- 2 Other-2 Substrate/Co-Factor Strip
- 1.5ml 5X Reaction Buffer A
- 25µl DTT, 0.1M

Cat.# V6930 ADP-Glo™ Kinase Assay, which includes:

- 2ml ADP-Glo™ Reagent
- 4ml Kinase Detection Reagent
- 500µl Ultra Pure ATP, 10mM
- 500µl ADP, 10mM

PRODUCT	SIZE	CAT.#
Kinase Selectivity Profiling System: Other-2	8 × 50 reactions	V6926

Kinase Selectivity Profiling System: Other-2 contains eight kinase/substrate pairs from the Other kinase family (see Figure 4). This system is sufficient for 50 sets of 8 kinase reactions (25 sets of 8 kinase reactions per strip pair) in 384-well plates using 2µl of Kinase Working Stock and 2µl of ATP/Substrate Working Stock. Includes:

- 2 Other-2 Kinase Strip
- 2 Other-2 Substrate/Co-Factor Strip
- 1.5ml 5X Reaction Buffer A
- 25µl DTT, 0.1M

PRODUCT	SIZE	CAT.#
Kinase Selectivity Profiling System: TKL-1 + ADP-Glo™ Assay	8 × 50 reactions	V6915

Kinase Selectivity Profiling System: TKL-1 + ADP-Glo™ Assay contains eight kinase/substrate pairs from the TKL kinase family (Figure 4). This system is sufficient for 50 sets of 8 kinase reactions (25 sets of 8 kinase reactions per strip pair) in 384-well plates using 2µl of Kinase Working Stock and 2µl of ATP/Substrate Working Stock. The system also includes sufficient ADP-Glo™ Assay reagents for 400 kinase assays. Includes:

Cat.# V6914 Kinase Selectivity Profiling System: TKL-1, which includes:

- 2 TKL-1 Kinase Strip
- 2 TKL-1 Substrate/Co-Factor Strip
- 1.5ml 5X Reaction Buffer A
- 25µl DTT, 0.1M

Cat.# V6930 ADP-Glo™ Kinase Assay, which includes:

- 2ml ADP-Glo™ Reagent
- 4ml Kinase Detection Reagent
- 500µl Ultra Pure ATP, 10mM
- 500µl ADP, 10mM



2. Product Components and Storage Conditions (continued)

PRODUCT	SIZE	CAT.#
Kinase Selectivity Profiling System: TKL-1	8 × 50 reactions	V6914

Kinase Selectivity Profiling System: TKL-1 contains eight kinase/substrate pairs from the TKL kinase family (Figure 4). This system is sufficient for 50 sets of 8 kinase reactions (25 sets of 8 kinase reactions per strip pair) in 384-well plates using 2 μ l of Kinase Working Stock and 2 μ l of ATP/Substrate Working Stock. Includes:

- 2 TKL-1 Kinase Strip
- 2 TKL-1 Substrate/Co-Factor Strip
- 1.5ml 5X Reaction Buffer A
- 25 μ l DTT, 0.1M

PRODUCT	SIZE	CAT.#
Kinase Selectivity Profiling System: STE-1 + ADP-Glo™ Assay	8 × 50 reactions	V6917

Kinase Selectivity Profiling System: STE-1 + ADP-Glo™ Assay contains eight kinase/substrate pairs from the STE kinase family (see Figure 4). This system is sufficient for 50 sets of 8 kinase reactions (25 sets of 8 kinase reactions per strip pair) in 384-well plates using 2 μ l of Kinase Working Stock and 2 μ l of ATP/Substrate Working Stock. The system also includes sufficient ADP-Glo™ Assay reagents for 400 kinase assays. Includes:

Cat.# V6916 Kinase Selectivity Profiling System: STE-1, which includes:

- 2 STE-1 Kinase Strip
- 2 STE-1 Substrate/Co-Factor Strip
- 1.5ml 5X Reaction Buffer A
- 25 μ l DTT, 0.1M

Cat.# V6930 ADP-Glo™ Kinase Assay, which includes:

- 2ml ADP-Glo™ Reagent
- 4ml Kinase Detection Reagent
- 500 μ l Ultra Pure ATP, 10mM
- 500 μ l ADP, 10mM

PRODUCT	SIZE	CAT.#
Kinase Selectivity Profiling System: STE-1	8 × 50 reactions	V6916

Kinase Selectivity Profiling System: STE-1 contains eight kinase/substrate pairs from the STE kinase family (see Figure 4). This system is sufficient for 50 sets of 8 kinase reactions (25 sets of 8 kinase reactions per strip pair) in 384-well plates using 2 μ l of Kinase Working Stock and 2 μ l of ATP/Substrate Working Stock. Includes:

- 2 STE-1 Kinase Strip
- 2 STE-1 Substrate/Co-Factor Strip
- 1.5ml 5X Reaction Buffer A
- 25 μ l DTT, 0.1M

PRODUCT	SIZE	CAT.#
Kinase Selectivity Profiling System: General Panel + ADP-Glo™ Assay	24 × 50 reactions	V6929

Kinase Selectivity Profiling System: General Panel + ADP-Glo™ Assay contains 24 kinases representative of the human kinome organized in strips of 8 kinase/substrate pairs (see Figure 4). This system is sufficient for 50 sets of 24 kinase reactions (25 sets of 8 kinase reactions per strip pair) in 384-well plates using 2µl of Kinase Working Stock and 2µl of ATP/Substrate Working Stock. The system also includes three ADP-Glo™ Assays (Cat.# V6930), sufficient for 1,200 kinase assays. Includes:

Cat.# V6928 Kinase Selectivity Profiling System: General Panel, which includes:

- 2 General Panel Kinase Strip #1
- 2 General Panel Kinase Strip #2
- 2 General Panel Kinase Strip #3
- 2 General Panel Substrate/Co-Factor Strip #1
- 2 General Panel Substrate/Co-Factor Strip #2
- 2 General Panel Substrate/Co-Factor Strip #3
- 3 × 1.5ml 5X Reaction Buffer A
- 3 × 25µl DTT, 0.1M

Three Cat.# V6930 ADP-Glo™ Kinase Assay, each of which includes:

- 2ml ADP-Glo™ Reagent
- 4ml Kinase Detection Reagent
- 500µl Ultra Pure ATP, 10mM
- 500µl ADP, 10mM

PRODUCT	SIZE	CAT.#
Kinase Selectivity Profiling System: General Panel	24 × 50 reactions	V6928

Kinase Selectivity Profiling System: General Panel contains 24 kinases representative of the human kinome organized in strips of 8 kinase/substrate pairs (see Figure 4). This system is sufficient for 50 sets of 24 kinase reactions (25 sets of 8 kinase reactions per strip pair) in 384-well plates using 2µl of Kinase Working Stock and 2µl of ATP/Substrate Working Stock. Includes:

- 2 General Panel Kinase Strip #1
- 2 General Panel Kinase Strip #2
- 2 General Panel Kinase Strip #3
- 2 General Panel Substrate/Co-Factor Strip #1
- 2 General Panel Substrate/Co-Factor Strip #2
- 2 General Panel Substrate/Co-Factor Strip #3
- 3 × 1.5ml 5X Reaction Buffer A
- 3 × 25µl DTT, 0.1M

Note: For each system, there are two separate inner packages, each of which contains a single Kinase Strip and the corresponding Substrate/Co-Factor Strip. The General Panel Kinase Selectivity Profiling Systems include six separate inner packages.

Storage Conditions: Store the Kinase Selectivity Profiling Systems below –65°C. Before use, thaw 5X Reaction Buffer A and 0.1M DTT at room temperature, and thaw the Substrate/Co-Factor Strip on ice. Immediately before use, thaw the Kinase Strip on ice, dilute and use immediately. After use, discard any remaining Kinase Working Stock and Substrate/Co-Factor Working Stock. Store any remaining 5X Reaction Buffer A and 0.1M DTT at –20°C for future use with the second Kinase Strip and Substrate/Co-Factor Strip.

2. Product Components and Storage Conditions (continued)

<p>TK-1</p> <ul style="list-style-type: none"> EGFR HER2 HER4 IGF1R InsR KDR PDGFRα PDGFRβ <ul style="list-style-type: none"> Poly (E,Y) Poly (E,Y) Poly (E,Y) IGF1Rtide Axltide Poly (E,Y) Poly (E,Y) Poly (E,Y) 	<p>TK-2</p> <ul style="list-style-type: none"> ABL1 BRK BTK CSK FYN A LCK LYN B SRC <ul style="list-style-type: none"> Abltide Poly (E,Y) Poly (E,Y) Poly (E,Y) Poly (E,Y) Poly (E,Y) SRC Substrate SRC Substrate 	<p>TK-3</p> <ul style="list-style-type: none"> AXL1 EPHA1 FAK ITK JAK3 PYK2 SYK TRKA <ul style="list-style-type: none"> Axltide Poly (E,Y) Poly (E,Y) Poly (E,Y) Poly (E,Y) Poly (E,Y) Poly (E,Y) Poly (E,Y)
<p>TK-4</p> <ul style="list-style-type: none"> c-MER FGFR1 FGFR2 FGFR4 FLT1 FMS MET RET <ul style="list-style-type: none"> Poly (E,Y) Poly (E,Y) Poly (E,Y) Poly (E,Y) IGF1Rtide Poly (E,Y) Poly (E,Y) IGF1Rtide 	<p>CMGC-1</p> <ul style="list-style-type: none"> ERK2 GSK3β JNK1 JNK3 p38α p38β p38δ p38γ <ul style="list-style-type: none"> MBP GSK3 Substrate p38 Substrate p38 Substrate p38 Substrate p38 Substrate p38 Substrate p38 Substrate 	<p>CMGC-2</p> <ul style="list-style-type: none"> CDK1/CyclinA2 CDK2/CyclinE1 CDK3/CyclinE1 CDK5/p25 CDK5/p35 CDK6/CyclinD3 CDK9/CyclinK CLK3 <ul style="list-style-type: none"> Histone H1 Protein Histone H1 Protein Histone H1 Protein Histone H1 Protein Histone H1 Protein Histone H1 Protein PDKtide MBP
<p>CAMK-1</p> <ul style="list-style-type: none"> CHK1 CHK2 MAPKAPK2 MARK1 MELK PASK PIM1 PKCμ (PKD1) <ul style="list-style-type: none"> CHKtide CHKtide HSP27tide CHKtide ZiPtide ZiPtide S6K Substrate CREBtide 	<p>CAMK-2</p> <ul style="list-style-type: none"> AMPK A1/B1/G1 AMPK A1/B1/G2 AMPK A2/B1/G1 CAMK2α CAMK2γ CAMK4 DAPK1 STK33 <ul style="list-style-type: none"> SAMStide SAMStide SAMStide Autocamtide-2 Autocamtide-2 Autocamtide-2 MBP MBP 	<p>STE-1</p> <ul style="list-style-type: none"> ASK1 HPK1 MINK1 MST1 NIK PAK1/CDC42 PAK3 TNIK <ul style="list-style-type: none"> MBP MBP MBP Axltide MBP PAKtide MBP MBP
<p>TKL-1</p> <ul style="list-style-type: none"> ALK2 ALK4 IRAK4 MLK2 RIPK2 TAK1-TAB2 TGFβRR2 ZAK <ul style="list-style-type: none"> Casein Casein MBP MBP MBP MBP MBP MBP 	<p>AGC-1</p> <ul style="list-style-type: none"> AKT1 p70S6Kβ PDK1 PKA PKC PRKG1 ROCK1 RSK2 <ul style="list-style-type: none"> AKT (PKB) Substrate RSK Substrate PDKtide Kemptide Neurogranin Peptide Substrate RSK Substrate S6K Substrate RSK Substrate 	<p>AGC-2</p> <ul style="list-style-type: none"> PKCα PKCβ II PKCδ PKCϵ PKCγ PKCζ PKCθ PKCζ <ul style="list-style-type: none"> CREBtide CREBtide CREBtide PKCtide PKCtide CREBtide PKCtide CREBtide
<p>Other/CK-1</p> <ul style="list-style-type: none"> Aurora A Aurora B CK2α1 DNA-PK CK1α1 CK1ϵ CK1γ1 VRK2 <ul style="list-style-type: none"> MBP MBP Casein DNA-PK Peptide Substrate De-Phospho Casein De-Phospho Casein Casein Casein 	<p>Other-2</p> <ul style="list-style-type: none"> CDC7/DBF4 EIF2AK2 IKKβ NEK2 NEK3 PLK1 TBK1 ULK1 <ul style="list-style-type: none"> PDKtide MBP IKKtide MBP MBP De-Phospho Casein MBP MBP 	
<p>General Panel</p>		
<p>GP-1</p> <ul style="list-style-type: none"> FGFR1 JAK3 LCK SYK MINK1 PAK1/CDC42 IRAK4 TAK1-TAB1 <ul style="list-style-type: none"> Poly (E,Y) Poly (E,Y) Poly (E,Y) Poly (E,Y) MBP PAKtide MBP MBP 	<p>GP-2</p> <ul style="list-style-type: none"> CDK2/CyclinE1 GSK3β p38α AMPK A1/B1/G2 CAMK4 CHK1 DAPK1 MAPKAPK2 <ul style="list-style-type: none"> Histone H1 Protein GSK3 Substrate p38 Substrate SAMStide Autocamtide-2 CHKtide MBP HSP27tide 	<p>GP-3</p> <ul style="list-style-type: none"> AKT1 PKCα ROCK1 Aurora A CK2α1 IKKβ CK1α1 CK1γ1 <ul style="list-style-type: none"> AKT (PKB) Substrate CREBtide S6K Substrate MBP Casein IKKtide De-Phospho Casein Casein

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Figure 4. (previous page) Kinase Selectivity Profiling System components. The kinases in the Kinase Strips and substrates in the Substrate/Co-Factor Strips are shown. Cofactors are listed in Table 6 in Section 9. Each kinase family is identified by a unique strip tube color; the Kinase Strip and corresponding Substrate/Co-Factor Strip share the same strip tube color. To distinguish between the Kinase and Substrate/Co-Factor Strips within the system, the Kinase Strips contain a blue dye and the Substrate/Co-Factor Strips contain a yellow dye at the same position. The dye is always located in one of the top four tubes of the strip, and the position of the dye is an indication of the strip name (e.g., the TK-3 Kinase Strip has dye in the third tube from the top position). The plastic strip tubes of the Kinase Selectivity Profiling Systems: General Panel, Kinase Selectivity Profiling Systems: Other/CK-1 and Kinase Selectivity Profiling Systems: Other-2 are clear. To differentiate the Kinase Strips and Substrate/Co-Factor Strips of these systems, we have included the blue and yellow dyes in two tubes. Poly (E,Y) is Poly (Glu₄, Tyr₁).

3. Before You Begin

Storage temperature: Kinases are temperature-sensitive and should be stored at -70°C until use. When using the Kinase Selectivity Profiling Systems, keep the Kinase Strip on ice during thawing and dilution steps. Minimize the time that the kinase spends at room temperature prior to reaction setup for optimal kinase activity. Some substrates are proteins, so you should avoid fluctuations in the storage temperature of the Substrate/Co-Factor Strips. Keep Substrate/Co-Factor Strips on ice during thawing and dilution steps. Substrates are less sensitive to storage temperature than kinases and can withstand longer periods at room temperature prior to kinase reaction setup and still maintain optimal kinase reaction activity.

Reaction temperature: For best results use an incubator or plate warmer at $22-25^{\circ}\text{C}$ for all incubations to minimize variability in assay temperature.

Plate recommendations: We recommend using solid-white, 384-well, low-volume plates suitable for luminescence measurements (e.g., Corning Cat.# 3674). Contact your luminometer manufacturer to determine which plates are best suited for your particular instrument.

Materials to Be Supplied By the User

- DMSO or compound vehicle of choice
- solid-white, 384-well plates compatible with the luminometer (do not use black or clear plates)
- 96-well plate for compound dilutions
- multichannel pipette or automated pipetting station capable of dispensing $1\mu\text{l}$ and $2\mu\text{l}$ volumes
- compound to be tested
- ADP-Glo™ Kinase Assay (Cat.# V6930), required for use with the Kinase Selectivity Profiling Systems Cat.# V6850, V6852, V6854, V6856, V6858, V6910, V6914, V6916, V6918, V6920, V6922, V6924, V6926, V6928 and V6932
- luminometer capable of reading multiwell plates, such as the GloMax® Discover Detection System (Promega Cat.# GM3000)
- plate shaker
- centrifuge compatible with a 384-well plate
- incubator or plate warmer at $22-25^{\circ}\text{C}$



4. Reagent Preparation

4.A. Preparing the ADP-Glo™ Kinase Assay Reagents

For more detailed protocols for the ADP-Glo™ Kinase Assay, see the *ADP-Glo™ Kinase Assay Technical Manual* #TM313, available at: www.promega.com/protocols/ and the Kinase Enzyme System protocol available at: www.promega.com/resources/protocols/technical-bulletins/101/kinase-enzyme-system-protocol/

Equilibrate the Kinase Detection Reagent and ADP-Glo™ Reagent to room temperature prior to use.

4.B. Preparing the Kinase Reaction Buffers

The volumes of reagents prepared here are sufficient for assays using one Kinase Strip and one Substrate/Co-Factor Strip. If both pairs of strips are used in Section 5, prepare twice the volume of each buffer.

4X Kinase Buffer

Prepare 900µl of 4X Kinase Buffer.

Component	Volume	Final Concentration
water	178.6µl	
5X Reaction Buffer A	720µl	4X
DTT, 0.1M	1.4µl	200µM
Total volume	900µl	

2.5X Kinase Buffer

Prepare 900µl of 2.5X Kinase Buffer.

Component	Volume	Final Concentration
water	338µl	
4X Kinase Buffer	562µl	2.5X
Total volume	900µl	

Note: This buffer is used to dilute the Kinase Strips and assemble the no-enzyme control reactions.

1X Kinase Buffer with 5% DMSO

1. Prepare 1ml of 1X Kinase Buffer with 5% DMSO.

Component	Volume	Final Concentration
water	700µl	
4X Kinase Buffer	250µl	1X
100% DMSO	50µl	5%
Total volume	1.0ml	

Notes:

1. This buffer is used for the compound serial dilution. If performing single-dose experiments, prepare a total volume of 100µl of 1X Kinase Buffer with 5% DMSO.
2. When using a different compound vehicle other than DMSO, modify this step by substituting the other vehicle for DMSO. If your compound is resuspended in water, use 1X Kinase Buffer (i.e., without DMSO) for your compound serial dilution.

100µM ATP Solution

1. Prepare 500µl of 100µM ATP Solution.

Component	Volume	Final Concentration
water	495µl	
Ultra Pure ATP, 10mM	5µl	100µM
Total volume	500µl	

Store the remaining Ultra Pure ATP at –20°C.

Note: Use only the Ultra Pure ATP provided with the ADP-Glo™ Assay. Other sources of ATP may contain ADP, which could result in high background values. Store the 10mM Ultra Pure ATP on ice while preparing the 100µM ATP Solution.

4.C. Preparing the Kinase Selectivity Profiling Strips

Substrate/Co-Factor Strip Preparation

Each tube within the Substrate/Co-Factor Strips will generate a 2.5X ATP/Substrate Working Stock. The working stock prepared from one Substrate/Co-Factor Strip is sufficient for 25 reactions at 2µl per reaction.

1. Thaw the Substrate/Co-Factor Strip on ice.
2. Using a multichannel pipette, add 15µl of 100µM ATP Solution (prepared in Section 4.B) to each tube of the Substrate/Co-Factor Strip. Mix by pipetting several times.
3. Store the ATP/Substrate Working Stock on ice until use.

Kinase Strip Preparation

Each tube within the Kinase Strips will generate a 2.5X Kinase Working Stock. The working stock prepared from one Kinase Strip is sufficient for 25 reactions at 2µl per reaction. Dilute the Kinase Strip immediately before use. Avoid long-term storage of the Kinase Working Stock on ice.

1. Immediately before use, thaw the Kinase Strip on ice.
2. Using a multichannel pipette, dilute each kinase in the Kinase Strip by adding 95µl of 2.5X Kinase Buffer to each tube of the Kinase Strip. Mix by gently pipetting 2–3 times.

Note: Use the Kinase Working Stock immediately after dilution. Do not freeze or reuse any portion of the Kinase Strip.

5. Kinase Profiling Protocols

Prior to performing the kinase-profiling experiments, prepare the reagents as described in Section 4. There are two general protocols for the Kinase Selectivity Profiling Systems: compound dose-response curve (Section 5.B) and compound single-dose assay (Section 5.C). The working stocks prepared from each Kinase Strip and Substrate/Co-Factor Strip pair provides sufficient volumes to perform either protocol as described.

5.A. Orienting the Kinase Strips on a Strip Holder

Prior to dispensing the Kinase Working Stock and ATP/Substrate Working Stock to the assay plate, position the Kinase Strips on a strip tube holder (e.g., a 96-well plate) such that kinase 1 is at the top of the strip (e.g., EGFR in TK1 and AKT1 in AGC-1). The strips are not labelled with the kinase names, so we built two types of identifications into the strip system: strip tube color and dye position. The color of the plastic strip tubes will help you identify the kinase family (see Figure 4). In addition, we included a noninterfering dye in each Kinase Strip and Substrate/Co-Factor Strip (blue and yellow, respectively) in a unique position to help you identify the top of the Kinase Strips where kinase 1 is located and the top of the Substrate/Co-Factor Strip where the corresponding substrate is located. Figure 4 describes the identification system.

When assembling the kinase reactions in Sections 5.B and 5.C, ensure that the orientation of the Substrate/Co-Factor Strip matches the orientation of the Kinase Strip. For example, align strips so that the kinase tube with blue dye and the substrate tube with the yellow dye are nearest the top row of the assay plate. This ensures that the appropriate substrate is used for each kinase (i.e., the substrate in tube 1 is used with the kinase in tube 1, the substrate in tube 2 is used with the kinase in tube 2, the substrate in tube 3 is used with the kinase in tube 3, etc.).

5.B. Compound Dose-Response Curve Using Kinase Selectivity Profiling Systems

The following setup describes the use of one Kinase Strip, one Substrate/Co-Factor Strip and one compound for duplicate measurements at each compound concentration. This setup can be adjusted to allow additional measurements in the same assay plate as described in the examples below.

Example 1: Two different kinase strips can be tested against one compound by dispensing compound dilutions to every row of the assay plate. Add the Kinase Working Stock for the first kinase strip to alternating rows (e.g., rows A, C, E, G, I, K, M, O), and add the Kinase Working Stock for the second kinase strip to the other rows (e.g., rows B, D, F, H, J, L, N, P).

Example 2: Two different compounds can be tested against two of the same strip family by dispensing compound dilutions to alternating rows of the assay plate (e.g., rows A, C, E, G, I, K, M and O contain compound 1, and rows B, D, F, H, J, L, N and P contain compound 2) and dispensing the Kinase Working Stock from both Kinase Strips of one system to blocks of two consecutive rows (e.g., kinase 1 to rows A and B, kinase 2 to rows C and D, kinase 3 to rows E and F, etc.).

Compound Serial Dilution

1. Add 50µl of 1X Kinase Buffer with 5% DMSO (or 5% vehicle) to wells A2–A12 of a 96-well plate. Do not add buffer to well A1. This is the compound serial dilution plate.

Note: To perform dose-response experiments with more than one compound, prepare an additional row for each compound. Add 50µl of 1X Kinase Buffer with 5% DMSO (or 5% vehicle) to wells of the additional rows of the plate. Do not add buffer to wells in column 1.

2. Prepare 100µl of Compound Solution. The compound concentration of the Compound Solution should be five times the highest desired compound concentration of the dose-response curve. For example, if you are using staurosporine as the compound and the highest final concentration in the curve is 10µM, prepare a 50µM staurosporine solution as follows.

Component	Volume	Final Concentration
water	70µl	
4X Kinase Buffer	25µl	1X
1mM staurosporine in DMSO	5µl	50µM
Total volume	100µl	

Note: The final concentration of DMSO is 5%.

5.B. Compound Dose-Response Curve Using Kinase Selectivity Profiling Systems (continued)

3. Add 100µl of Compound Solution to well A1 of the compound serial dilution row (row A) of the compound serial dilution plate prepared in Step 1. Prepare a 10-point 1:2 or 1:4 serial dilution of your compound across the compound serial dilution row. Mix well by pipetting before each transfer to the next well.

An example of a 1:2 compound serial dilution is shown in Table 1. Other dilution factors can be used, depending on your needs.

Note: When using more than one compound, repeat this step for each compound using a different row of the plate.

Table 1. Preparing a 1:2 Serial Dilution of Compound.

Well Number	Starting Volume in Each Well	Volume of Compound to Transfer	Final Compound Concentration (nM)
A1	100µl	50µl to well A2	10,000
A2	50µl	50µl to well A3	5,000
A3	50µl	50µl to well A4	2,500
A4	50µl	50µl to well A5	1,250
A5	50µl	50µl to well A6	625
A6	50µl	50µl to well A7	312.5
A7	50µl	50µl to well A8	156.3
A8	50µl	50µl to well A9	78.1
A9	50µl	50µl to well A10	39.1
A10	50µl	0µl	19.5
A11 ¹	50µl	0µl	0
A12 ²	50µl	0µl	0

¹Well A11 is the no-compound control well.

²Well A12 is the no-enzyme control well.

Kinase Reaction Setup

Prior to dispensing the Kinase Working Stocks and ATP/Substrate Working Stocks to the assay plate, ensure that the orientation of the Substrate/Co-Factor Strip matches the orientation of the Kinase Strip by aligning the indicator dyes in the strips (see Section 5.A).

- Using a multichannel pipette or automated pipetting station, transfer 1 μ l of each compound serial dilution from the compound serial dilution row of the 96-well plate to each well in duplicate columns of alternating rows (i.e., rows A, C, E, G, I, K, M, O) of a 384-well plate. Do not add compound to the remaining rows (i.e., rows B, D, F, H, J, L, N, P). This is your assay plate, and rows A, C, E, G, I, K, M, O are the assay rows. See Step 1 in Figure 5.

The net result is that wells A1, A2, C1, C2, E1, E2, G1, G2, I1, I2, K1, K2, M1, M2, O1 and O2 contain 1 μ l of the compound dilution from well A1 of the compound serial dilution row; wells A3, A4, C3, C4, E3, E4, G3, G4, I3, I4, K3, K4, M3, M4, O3 and O4 contain 1 μ l of the compound dilution from well A2 of the compound serial dilution row; wells A5, A6, C5, C6, E5, E6, G5, G6, I5, I6, K5, K6, M5, M6, O5 and O6 contain 1 μ l of the compound dilution from well A3 of the compound serial dilution row; etc.

Note: Wells in columns 21 and 22 contain the no-compound controls and represent 100% kinase activity. Wells in columns 23 and 24 contain the no-enzyme controls and represent background luminescence.

- Transfer 2 μ l of 2.5X Kinase Buffer to wells 23 and 24 of the assay rows of the assay plate. These wells are the no-enzyme controls (see Step 2 in Figure 5).

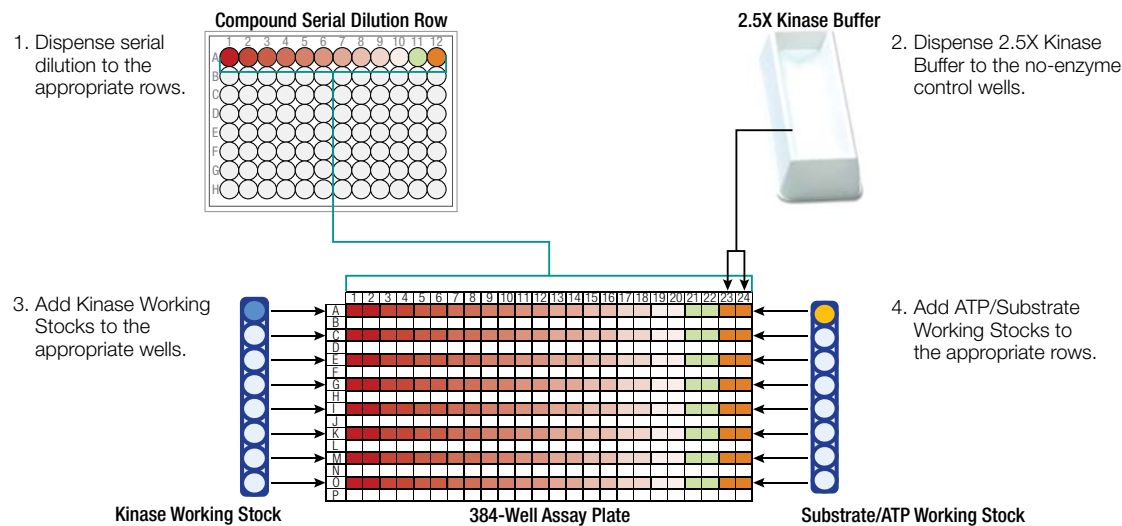


Figure 5. Experimental setup for creating a compound dose-response curve using the Kinase Selectivity Profiling Systems.

5.B. Compound Dose-Response Curve Using Kinase Selectivity Profiling Systems (continued)

- Transfer 2 μ l of the Kinase Working Stocks to wells 1 through 22 of every assay row of the assay plate (see Step 3 in Figure 5).

The net result is that wells A1–A22 contain 2 μ l of kinase 1 of the Kinase Strip; wells C1–C22 contain 2 μ l of kinase 2; wells E1–E22 contain 2 μ l of kinase 3; wells G1–G22 contain 2 μ l of kinase 4; wells I1–I22 contain 2 μ l of kinase 5; wells K1–K22 contain 2 μ l of kinase 6; wells M1–M22 contain 2 μ l of kinase 7 and wells O1–O22 contain 2 μ l of kinase 8.

- Centrifuge the assay plate briefly to bring reagents to the bottom of the wells. Mix with a plate shaker for 2 minutes. Incubate at room temperature (22–25°C) for 10 minutes.
- Transfer 2 μ l of ATP/Substrate Working Stocks to all wells of the assay rows, making sure to use the appropriate substrate for each kinase.
- Centrifuge the assay plate briefly to bring reagents to the bottom of the wells. Mix with a plate shaker for 2 minutes. Incubate at room temperature for 60 minutes.
- Proceed to Section 5.D to detect ADP.

5.C. Single-Dose Inhibition Profile Using Kinase Selectivity Profiling Systems

The following setup describes the use of one Kinase Strip, one Substrate/Co-Factor Strip and one compound for duplicate measurements at a single compound concentration and for the appropriate controls. This setup can be modified to allow additional measurements with up to ten compounds. When testing additional compounds, assemble an additional column of kinase reactions for each additional compound (e.g., use column 4 for a second compound, column 5 for a third compound, etc.). Up to 20 compounds can be tested against each kinase family in a single 384-well plate by using both Kinase Strips and Substrate/Co-Factor Strips of a single kit.

Preparation of Compound Solutions

Prepare 100 μ l of the Compound Solution in 1X Kinase Buffer. The compound concentration of the Compound Solution should be five times the desired final concentration. For example, if you are using staurosporine as the compound and kinase profiling will be performed at 1 μ M, prepare a 5 μ M staurosporine solution as follows.

Component	Volume	Final Concentration
water	70 μ l	
4X Kinase Buffer	25 μ l	1X
100 μ M staurosporine in DMSO	5 μ l	5 μ M
Total volume	100μl	

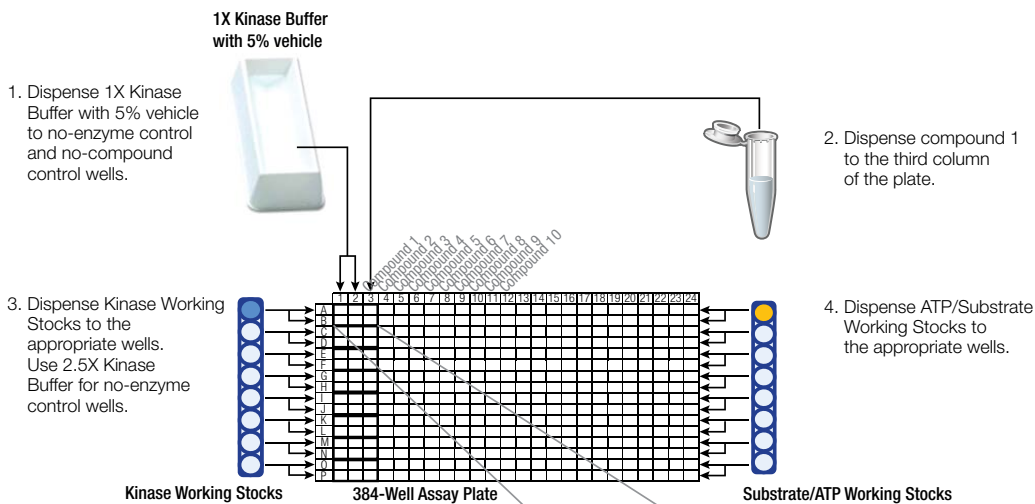
Note: The final concentration of DMSO is 5%.

Kinase Reaction Setup

Prior to dispensing the Kinase Working Stocks and ATP/Substrate Working Stocks to the assay plate, ensure that the orientation of the Substrate/Co-Factor Strip matches the orientation of the Kinase Strip by aligning the indicator dyes in the strips (see Section 5.A).

1. Transfer 1 μ l of 1X Kinase Buffer with 5% DMSO to each well of column 1 and 2 of a 384-well assay plate (see Step 1 of Figure 6).

Note: Wells in column 1 contain the no-enzyme control reactions and represent background luminescence. Wells in column 2 contain the no-compound control reactions and represent 100% kinase activity.



Well composition for complete single-point assay

Wells A1 and B1: 1X Kinase Buffer with 5% vehicle + 2.5X Kinase Buffer + ATP/Substrate Working Stocks
(No-enzyme control reactions)

Wells A2 and B2: 1X Kinase Buffer with 5% vehicle + Kinase Working Stocks + ATP/Substrate Working Stocks
(No-compound control reactions)

Wells A3 and B3: Compound 1 + Kinase Working Stocks + ATP/Substrate Working Stocks
(Kinase and compound reactions)

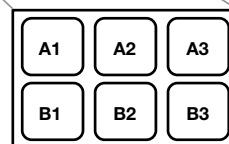


Figure 6. Experimental setup for creating a single-dose compound profile using Kinase Selectivity Profiling Systems.

5.C. Single-Dose Inhibition Profile Using Kinase Selectivity Profiling Systems (continued)

2. Transfer 1 μ l of the Compound Solution to all wells in column 3. This column contains the compound-containing reactions (see Step 2 of Figure 6).

Note: Columns 1, 2, and 3 are the assay columns. When profiling additional compounds, use an additional column of kinase reactions for each additional compound (e.g., use column 4 for a second compound, column 5 for a third compound, etc.).

3. Transfer 2 μ l of 2.5X Kinase Buffer to each well of column 1 of the assay plate.
4. Add 2 μ l of Kinase Working Stocks to all wells of columns 2 and 3, adding each kinase to two consecutive rows (see Step 3 of Figure 6).

The net result is that wells A2, A3, B2 and B3 contain 2 μ l of the Kinase Working Stock from the first tube of the Kinase Strip; wells C2, C3, D2 and D3 contain 2 μ l of the Kinase Working Stock from the second tube of the Kinase Strip; wells E2, E3, F2 and F3 contain 2 μ l of the Kinase Working Stock from the third tube of the Kinase Strip; etc.

Note: When testing additional compounds, be sure to add Kinase Working Stock to wells in each additional column.

5. Centrifuge the assay plate briefly to bring reagents to the bottom of the wells. Mix with a plate shaker for 2 minutes. Incubate at room temperature (22–25°C) for 10 minutes.
6. Transfer 2 μ l of ATP/Substrate Working Stocks to wells in all assay columns, adding each ATP/Substrate Working Stock to two consecutive rows and making sure to use the appropriate substrate for each kinase (see Step 4 of Figure 6).

The net result is that wells A1, A2, A3, B1, B2 and B3 contain 2 μ l of the ATP/Substrate Working Stock from the first tube of the Substrate/Co-Factor Strip; wells C1, C2, C3, D1, D2 and D3 contain 2 μ l of the ATP/Substrate Working Stock from the second tube of the Substrate/Co-Factor Strip; wells E1, E2, E3, F1, F2 and F3 contain 2 μ l of the ATP/Substrate Working Stock from the third tube of the Substrate/Co-Factor Strip; etc.

Note: When testing additional compounds, be sure to add ATP/Substrate Working Stock to wells in each additional column.

7. Centrifuge the assay plate briefly to bring the reagents to the bottom of the wells. Mix with a plate shaker for 2 minutes. Incubate at room temperature for 60 minutes.
8. Proceed to Section 5.D to detect ADP.

5.D. ADP Detection with ADP-Glo™ Kinase Assay

1. After the incubation in Section 5.B or 5.C, add 5 μ l of ADP-Glo™ Reagent to all reactions in the assay plate. Mix for 2 minutes, and incubate the reaction at room temperature for 40 minutes.
2. Add 10 μ l of Kinase Detection Reagent to all assay wells in the assay plate. Mix for 2 minutes, and incubate reactions at room temperature for 30 minutes.
3. Measure luminescence using an integration time of 0.5 seconds per well.

6. Data Interpretation

This section provides data-processing suggestions and short explanations of results that may be obtained.

Any data-fitting and analysis software can be used to analyze results generated using the Kinase Selectivity Profiling Systems. Kinase Selectivity Profiling Systems (KPS) SMART Protocols are available for data analysis and can be found at: www.promega.com/resources/tools/. Separate tools are available for dose-response and single-dose data. Raw data are imported or copied into the tool, and the IC₅₀ and % activity values are automatically calculated.

Kinase Selectivity Profiling Systems have been integrated with the Promega GloMax[®] Discover System for a streamlined workflow. The KPS SMART Protocol for use with the GloMax[®] Discover System includes automated IC₅₀ and percent activity calculations and can be found at: www.promega.com/resources/tools/detection-instruments-software/. The KPS SMART Protocol also can be obtained by contacting Promega Technical Services.

- **Calculate net luminescence:** Subtract the average no-enzyme control luminescence from all kinase-containing reactions with or without compound.
- **Convert net luminescence to percent activity values:** Using net luminescence of the no-compound control reactions to represent 100% kinase activity, calculate the percent kinase activity in the compound-containing reactions.

Potential Outcomes

1. Kinase reaction with compound produced a lower percent kinase activity than the no-compound control reaction:
Kinase + compound % kinase activity < no-compound control % kinase activity

Compound inhibited the kinase, as seen by the lower percent kinase activities in the test wells when compared to the no-compound control percent kinase activities. For example, gefitinib inhibits HER4 kinase, as shown in Table 2.

Table 2. HER4 Kinase Activity in the Presence of 1 μ M Gefitinib in a Single-Point Assay.

	Reactions with K + C + S ¹	Reactions with K + S	Reactions with S ²
Luminescence of replicate 1 (RLU)	6,134	21,157	475
Luminescence of replicate 2 (RLU)	6,668	21,839	519
Average luminescence (RLU)	6,401	21,498	497
Background-subtracted (net) luminescence (RLU)	5,904	21,001	0
HER4 activity (%)	28	100	0

¹K = kinase, C = compound, S = substrate

²No-enzyme control reactions were used to determine background luminescence.

6. Data Interpretation (continued)

2. The percent kinase activity of kinase reactions with compound is approximately equal to that of the no-compound control reaction:

$$\text{Kinase + compound \% kinase activity} = \text{no-compound control \% kinase activity}$$

Compound had no effect on kinase activity. For example, dasatinib has no effect on ITK kinase, as shown in Table 3.

Table 3. IKT Kinase Activity in the Presence of 1 μ M Dasatinib in a Single-Point Assay.

	Reactions with K + C + S ¹	Reactions with K + S	Reactions with S ²
Luminescence of replicate 1 (RLU)	24,615	25,093	271
Luminescence of replicate 2 (RLU)	23,607	22,903	291
Average luminescence (RLU)	24,111	23,998	281
Background-subtracted (net) luminescence (RLU)	23,830	23,717	0
ITK activity (%)	100	100	0

¹K = kinase, C = compound, S = substrate

²No-enzyme control reactions were used to determine background luminescence.

3. Kinase reaction with compound produced a higher percent kinase activity value than the no-compound control reaction:

$$\text{Kinase + compound \% kinase activity} > \text{no-compound control \% kinase activity}$$

The compound is an activator of the kinase reaction, or there were dispensing issues during plate setup. Check for discrepancies between replicate values, and ensure that the correct dispensing, centrifuging and mixing steps were performed during assay setup. If the ADP-Glo™ Reagent and kinase reaction were not mixed well, some ATP might remain on the sides of the well, creating higher luminescence.

4. Kinase reaction with compound produced a negative percent kinase activity value:

$$\text{Kinase + compound \% kinase activity} = \text{negative value}$$

Subtracting high luminescence values for the no-enzyme control reactions from the experimental data can cause the percent kinase activity to be less than zero. Check for high background values, and ensure that correct dispensing, centrifuging and mixing steps were performed during assay setup.

7. Composition of Buffers and Solutions

5X Reaction Buffer A

200mM	Tris-HCl (pH 7.5)
100mM	MgCl ₂
0.5mg/ml	BSA

8. References

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9. Substrate and Cofactor Information

Tables 4–6 list the concentrations of substrates and cofactors in the Kinase Selectivity Profiling Systems.

Table 4. Concentration of Peptide Substrates as Supplied in the Substrate/Co-Factor Strips.

Peptide Substrate	Concentration
Abltide	0.66mg/ml
AKT (PKB) Substrate	0.66mg/ml
Autocamtide-2	0.66mg/ml
Axltide	0.66mg/ml
CHKtide	0.66mg/ml
CREBtide	0.66mg/ml
DNA-PK Peptide Substrate	0.66mg/ml
GSK3 Substrate	0.66mg/ml
HSP27tide	0.66mg/ml
IGF1Rtide	0.66mg/ml
IKKtide	0.66mg/ml
Kemptide	0.66mg/ml
Neurogranin Peptide Substrate	0.66mg/ml
p38 Substrate	0.66mg/ml
PAKtide	0.66mg/ml
PDKtide	0.66mg/ml
PKCtide	0.66mg/ml
Poly (Glu ₄ , Tyr ₁)	0.66mg/ml
RSK Substrate	0.66mg/ml
S6K Substrate	0.66mg/ml
SAMStide	0.66mg/ml
SRC Substrate	0.66mg/ml
ZIPTide	0.66mg/ml

Table 5. Concentration of Protein Substrates as Supplied in the Substrate/Co-Factor Strips.

Protein Substrate	Concentration
Casein	0.33mg/ml
De-Phospho Casein	0.33mg/ml
Histone H1 Protein	0.33mg/ml
MBP	0.33mg/ml

Table 6. Concentration of Cofactors as Supplied in the Substrate/Co-Factor Strips.

Cofactor	Concentration	Kinase Reaction
AMP	1.66 μ M	AMPK A1/B1/G1, AMPK A1/B1/G2, AMPK A2/B1/G1
Ca ²⁺ /Calmodulin	1.66mM CaCl ₂ 0.1 μ g/ μ l calmodulin	CAMK2 α , CAMK2 γ , CAMK4, DAPK1
cGMP	33.3 μ M	PRKG1
DNA Activator	33 μ M calf thymus DNA	DNA-PK
GTP	333.3 μ M	PAK1/CDC42
MnCl ₂	6.66mM	BRK, BTK, c-MER, CSK, EGFR, EPHA1, FAK, FGFR2, FGFR4, FLT1, FMS, HER4, IGF1R, InsR, ITK, LCK, LYN B, PYK2, SRC
PKC Lipid Activator	166 μ g/ml phosphatidylserine 16.66 μ g/ml 1-stearoyl-2-linoleoyl-sn-glycerol 16.66 μ g/ml 1-oleoyl-2-acetyl-sn-glycerol	PKC, PKC α , PKC β II, PKC δ , PKC ϵ , PKC γ , PKC ζ , PKC θ



10. Related Products

Kinase Assays

Product	Size	Cat.#
ADP-Glo™ Kinase Assay	400 assays	V6930
	1,000 assays	V9101
	10,000 assays	V9102
	100,000 assays	V9103
ADP-Glo™ Max Assay	1,000 assays	V7001
	10,000 assays	V7002
Kinase-Glo® Luminescent Kinase Assay*	10ml	V6711
Kinase-Glo® Plus Luminescent Kinase Assay*	10ml	V3771
Kinase-Glo® Max Luminescent Kinase Assay*	10ml	V6071
ProFluor® PKA Assay*	4 plate	V1240
ProFluor® Src-Family Kinase Assay*	4 plate	V1270
PepTag® Non-Radioactive PKC Assay	120 reactions	V5330
PepTag® Non-Radioactive cAMP-Dependent Protein Kinase Assay	120 reactions	V5340

*Additional sizes available.

Kinase Enzyme Systems

Product	Size	Cat.#
GSK3 Beta Kinase Enzyme System	10µg	V1991
BTK Kinase Enzyme System	10µg	V2942
CDK5/p35 Kinase Enzyme System	10µg	V3271
EGFR Kinase Enzyme System	10µg	V3831
NIK Kinase Enzyme System	10µg	V4076
DNA-PK Kinase Enzyme System	2,500 units	V4106
V4506 EGFR (T790M) Kinase Enzymes System	10µg	V4506

More than 170 Kinase Enzyme Systems (KES) are available from Promega Corporation. Each system is optimized for use with the ADP-Glo™ Kinase Assay. Each KES contains enzyme, substrate, reaction buffer and any supplemental components needed to run the assay. Visit www.promega.com/kinase/ for more information.

Lipid Kinase Reagents

Product	Size	Cat.#
PI3K-Glo™ Class I Profiling Kit	1 each	V1690
PIP2:3PS Lipid Kinase Substrate, 0.25mg	0.25ml	V1701
PI:3PS Lipid Kinase Substrate, 0.5mg	0.5ml	V1711
PI3K (p110α/p85α), 20μg	200μl	V1721
PI3K (p110β/p85α), 20μg	200μl	V1751
PI3K (p120γ), 20μg	200μl	V1761
PI3K (p110δ/p85α), 20μg	200μl	V1771
ADP-Glo™ Kinase Assay with PI:3PS	1,000 assays	V1781
	10,000 assays	V1782
ADP-Glo™ Kinase Assay with PIP2:3PS	1,000 assays	V1791
	10,000 assays	V1792

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

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Luminometers

Product	Size	Cat.#
GloMax® Discover System	1 each	GM3000

11. Summary of Changes

The following changes were made to the 7/15 revision of this document:

1. Corrected Cat.# V6831 to V6931 on page 10.



^(a)U.S. Pat. Nos. 7,083,911, 7,452,663 and 7,732,128, European Pat. No. 1383914 and Japanese Pat. Nos. 4125600 and 4275715.

^(b)U.S. Pat. No. 7,700,310, European Pat. No. 1546374 and other patents pending.

^(c)U.S. Pat. Nos. 7,741,067, 8,361,739, 8,603,767, Japanese Pat. No. 4485470 and other patents pending.

^(d)U.S. Pat. Nos. 6,602,677, 7,241,584 and 8,030,017, European Pat. No. 1131441, Japanese Pat. Nos. 4537573 and 4520084 and other patents pending.

^(e)U.S. Pat. No. 8,183,007 and other patents and patents pending.

^(f)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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