



## ADP-Glo™ Kinase Profiling Application Notes

# TYROSINE KINASE KSPS: TK-4

# Kinase Selectivity Profiling System: TK-4

By Jacquelyn Hennek, M.S., Said A. Goueli, Ph.D., and Hicham Zegzouti, Ph.D., Promega Corporation

### Scientific Background:

Kinase Selectivity Profiling System TK-4 is a set of kinases from the Tyrosine Kinase Family presented in an easy to use 8-tube strip format. When diluted, the kinase stock volumes are standardized to generate optimal ATP to ADP conversion with a signal to background ratio over 10-fold when their activities are detected using the ADP-Glo™ Kinase Assay (Fig. 1). The substrate stocks are standardized in a similar fashion and are located in a second strip at corresponding positions. Kinase Selectivity Profiling Systems can be used to generate single-dose inhibitor selectivity profiles for as many inhibitors as desired (Fig. 2A) or to study dose response curves for an inhibitor (Fig. 2B).

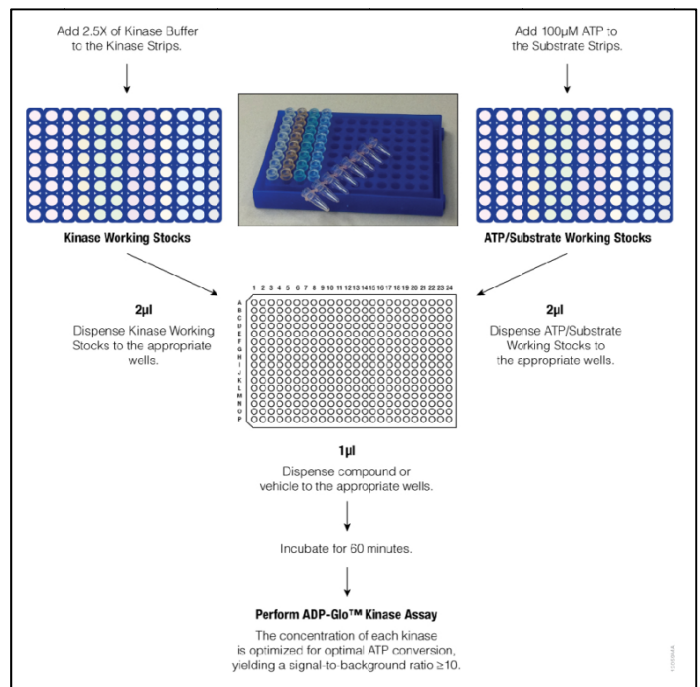
TK-4		
TK Family		
	Kinase Strip	Substrate Strip
A	c-MER	Poly (Glu <sub>4</sub> , Tyr <sub>1</sub> ) + MnCl <sub>2</sub>
B	FGFR1	Poly (Glu <sub>4</sub> , Tyr <sub>1</sub> )
C	FGFR2	Poly (Glu <sub>4</sub> , Tyr <sub>1</sub> ) + MnCl <sub>2</sub>
D	FGFR4	Poly (Glu <sub>4</sub> , Tyr <sub>1</sub> ) + MnCl <sub>2</sub>
E	FLT1	IGF1Rtide + MnCl <sub>2</sub>
F	FMS	Poly (Glu <sub>4</sub> , Tyr <sub>1</sub> ) + MnCl <sub>2</sub>
G	MET	Poly (Glu <sub>4</sub> , Tyr <sub>1</sub> )
H	RET	IGF1Rtide

### ADP-Glo™ Kinase Assay

#### Description

ADP-Glo™ Kinase Assay is a luminescent kinase assay that measures ADP formed from a kinase reaction; ADP is converted into ATP, which is converted into light by Ultra-Glo™ Luciferase.

The luminescent signal positively correlates with ADP amount and kinase activity. The assay is well suited for measuring the effects chemical compounds have on the activity of a broad range of purified kinases—making it ideal for both primary screening as well as kinase selectivity profiling (Fig. 2).



**Figure 1. Kinase Selectivity Profiling System Overview.** Kinases are provided at either 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 into the strips produce sufficient Kinase and ATP/Substrate Working Stocks for 25 kinase reactions. Kinase reactions are performed using 1µL of compound, 2µL of Kinase Working Stock, and 2µL of ATP/Substrate Working Stock. After 1 hour incubation at room temperature, 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 correlated with kinase activity.

For detailed protocols on strip preparation, single-dose inhibition profiles, and creating dose-response curves, see *The Kinase Selectivity Profiling System* Technical Manual #TM421, available at [www.promega.com/protocols/tm421](http://www.promega.com/protocols/tm421)



#### Preparation of Kinase and ATP/Substrate Working Stocks:

- Add 95µl of 2.5X Kinase Buffer to all tubes in the Kinase Strip.
- Add 15µl of 100µM ATP to all tubes in the Substrate/Cofactors Strip.

#### Single-Dose Inhibition Profile:

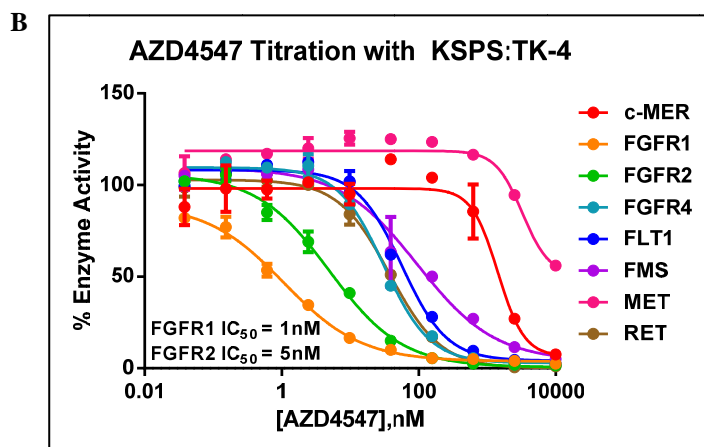
- Setup Kinase Reactions and No Compound Controls:
  - 1µl of compound or vehicle (5% DMSO)
  - 2µl of Kinase Working Stock
- Setup No Kinase Controls:
  - 1µl vehicle (5% DMSO)
  - 2µl of Kinase Buffer
- Incubate at room temperature for 10 minutes.
- Add 2µl of ATP/Substrate Working Stock.
- Incubate at room temperature for 60 minutes.
- Perform ADP detection using ADP-Glo™ Kinase Assay.

#### Dose-Response Curves:

- Setup Kinase Reactions:
  - 1µl of 5X compound serial dilution
  - 2µl of Kinase Working Stock
- Setup No Kinase Controls:
  - 2µl of Kinase Buffer in place of Kinase Working Stock
- Incubate at room temperature for 10 minutes.
- Add 2µl of ATP/Substrate Working Stock.
- Incubate at room temperature for 60 minutes.
- Perform ADP detection using ADP-Glo™ Kinase Assay.

A

		Gefitinib	AZD4547
<b>KSPS: TK-4</b>	<b>c-MER</b>	71	72
	<b>FGFR1</b>	58	0
	<b>FGFR2</b>	103	1
	<b>FGFR4</b>	100	5
	<b>FLT1</b>	100	8
	<b>FMS</b>	102	22
	<b>MET</b>	91	94
	<b>RET</b>	71	3



**Figure 2. KSPS: TK-4 Profiling Data.** (A) KSPS: TK-4 kinase activities were determined in the presence of 1µM Gefitinib or AZD4547. % Activity values were calculated using No Compound and No Kinase Controls and are shown above. Red < 20%; White 20-60%; Blue > 60%. (B) AZD4547 dose response curves were created with KSPS: TK-4 to determine the potency (IC<sub>50</sub>) and selectivity of the inhibitor. IC<sub>50</sub> values are comparable to literature values <sup>(1)</sup>.

<sup>(1)</sup> Gavine, P. R. et. al.; Cancer Res, 2013, 72, 2045.

#### Assay Components and Ordering Information:



Products	Company	Cat.#
ADP-Glo™ Kinase Assay	Promega	V6930
Kinase Selectivity Profiling System: TK-4	Promega	V6922
Kinase Selectivity Profiling System: TK-4 + ADP-Glo™ Assay	Promega	V6923

Kinase Buffer: 40mM Tris, pH 7.5; 20mM MgCl<sub>2</sub>; 0.1mg/ml BSA; 50µM DTT.