ADP-Glo™ Kinase Assay Application Note

Tyrosine Kinase Series

EML4-ALK Kinase Assay

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Scientific Background:

ALK or CD246 is a receptor tyrosine kinase, which belongs to the insulin receptor superfamily which comprises an extracellular domain, a hydrophobic stretch corresponding to a single pass transmembrane region, and an intracellular kinase domain. CD246 plays an important role in the development of the brain and exerts its effects on specific neurons in the nervous system. ALK-positive neoplasms represent a distinct entity because the morphology of the tumors is often neither anaplastic nor large cell and the tumors should be referred to as ALK lymphomas (1). CD246 has been found to be rearranged, mutated, or amplified in a series of tumors including anaplastic large cell lymphomas, neuroblastoma, and non-small cell lung cancer. EML4-ALK mutations in lung cancer confer resistance to ALK inhibitors (2).

1. Benharroch, D. et. al: ALK-positive lymphoma: a single disease with a broad spectrum of morphology. Blood 91: 2076-2084, 1998.
2. Choi, Y. L. et.al: EML4-ALK mutations in lung cancer that confer resistance to ALK inhibitors. New Eng. J. Med. 363: 1734-1739, 2010.

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 (Fig. 1). The luminescent signal positively correlates with ADP amount (Fig. 2) and kinase activity (Fig. 3A). 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. 3B). The ADP-Glo™ Kinase Assay can be used to monitor the activity of virtually any ADP-generating enzyme (e.g., kinase or ATPase) using up to 1mM ATP.

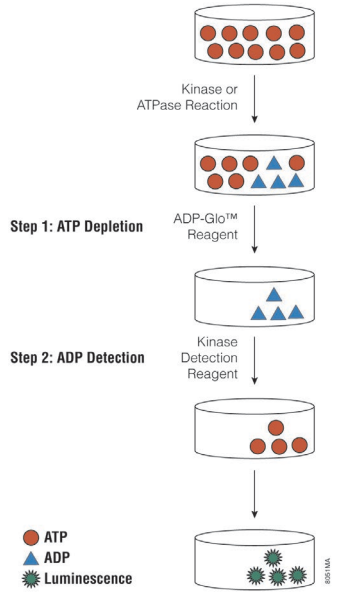

Figure 1. Principle of the ADP-Glo™ Kinase Assay. The ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.

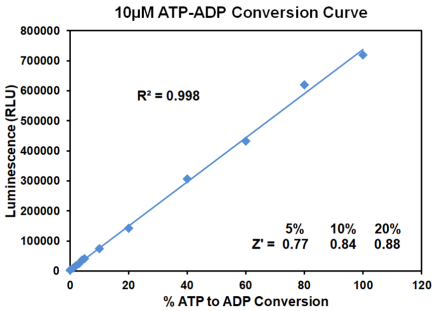

Figure 2. Linearity of the ADP-Glo Kinase Assay. ATP-to-ADP conversion curve was prepared at 10µM ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction. Z' factors were determined using 200 replicates of each of the % conversions shown.

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The following is only a short protocol. For detailed protocols on conversion curves, kinase assays and inhibitor screening, see Kinase Enzyme Systems Protocol at: http://www.promega.com/KESProtocol

Short Protocol

- Dilute enzyme, substrate, ATP and inhibitors in 1x kinase reaction buffer.
- Add to the wells of 384 low volume plate:
  - ✓ 1 µl of inhibitor or (5% DMSO)
  - ✓ 2 µl of enzyme (defined from table 1)
  - ✓ 2 µl of substrate/ATP mix
- Incubate at room temperature for indicated time (See Figure 3).
- Add 5 µl of ADP-Glo™ Reagent.
- Incubate at room temperature for 40 minutes.
- Add 10 µl of Kinase Detection Reagent.
- Incubate at room temperature for 30 minutes.
- Record luminescence (Integration time 0.5-1 second).

Table 1. Enzyme Titration. Data are shown as relative light units (RLU) that directly correlate to the amount of ADP produced. The correlation between the % of ATP converted to ADP and corresponding signal to background ratio is indicated for each kinase amount.

Enzyme, ng	150	75	37.50	18.75	9.38	4.69	2.34	0
Luminescence	159,881	54,693	27,712	19,131	10,191	5,778	3,622	1,988
S/B	80	28	14	10	5	3	2	1
% Conversion	22	7	4	2	1	1	0	0

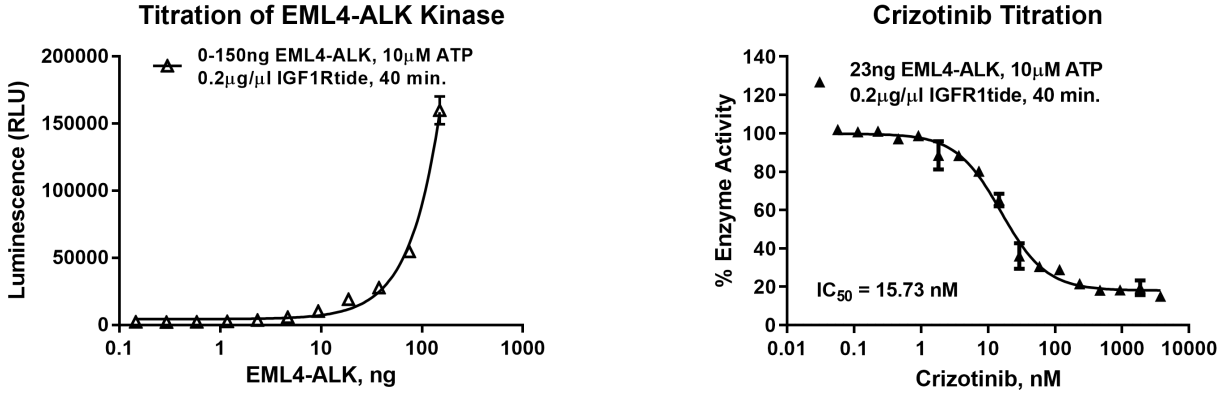

Figure 3. EML4-ALK Kinase Assay Development. (A) EML4-ALK enzyme was titrated using 10µM ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) Inhibitor dose response was created using 23ng of EML4-ALK to determine the potency of the inhibitor (IC_{50}).

Ordering Information:

Products	Size	Cat. #
EML4-ALK Kinase Enzyme System	10µg	VA7357
	1mg	VA7358
ADP-Glo™ + EML4-ALK Kinase Enzyme System	1 Each	VA7359