

ACK Kinase Assay

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Scientific Background:

ACK is a tyrosine kinase that binds CDC42Hs in its GTP-bound form and inhibits both the intrinsic and GTPase-activating protein (GAP)-stimulated GTPase activity of CDC42Hs (1). Overexpression of ACK in cancer cell lines of epithelial origin increases cellular motility and invasiveness. In a mouse model, ACK overexpression enhances the ability of a human breast cancer cell line to metastasize to the lung and increased mortality (2). Ligand stimulation of alpha-3 beta-1 integrin leads to activation of ACK which then enhances p130CAS phosphorylation and activation of RAC.

1. Manser, E. et al: A non-receptor tyrosine kinase that inhibits the GTPase activity of p21cdc42. *Nature* 363: 364-367, 1993.
2. van der Horst, E. H.; Metastatic properties and genomic amplification of the tyrosine kinase gene ACK1. *Proc. Nat. Acad. Sci.* 102: 15901-15906, 2005.

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.

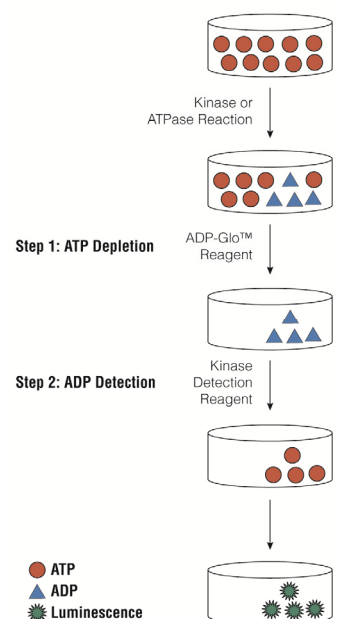


Figure 1. Principle of the ADP-Glo™ Kinase Assay. The ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.

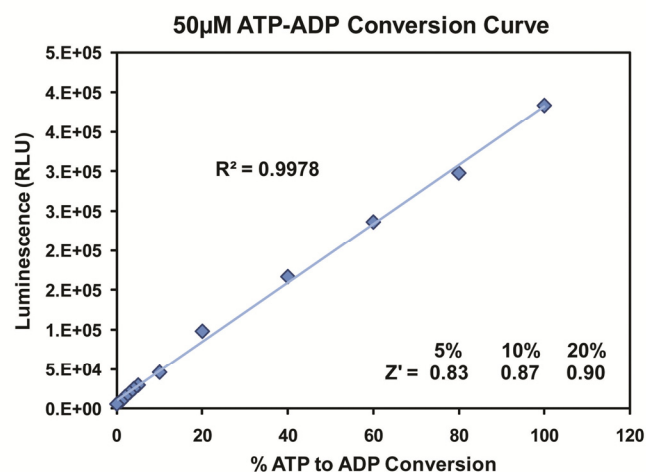
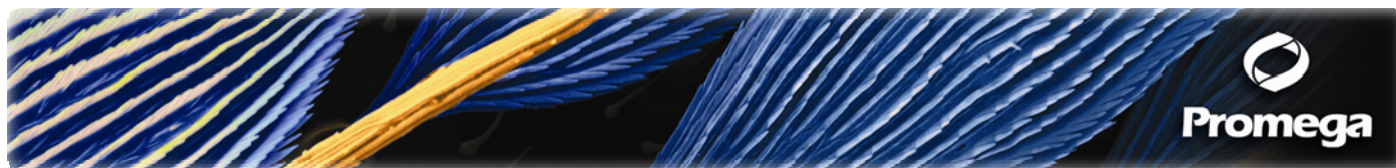


Figure 2. Linearity of the ADP-Glo Kinase Assay. ATP-to-ADP conversion curve was prepared at 50µM ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction. Z' factors were determined using 200 replicates of each of the % conversions shown.



For detailed protocols on conversion curves, kinase assays and inhibitor screening, see *The ADP-Glo™ Kinase Assay Technical Manual #TM313*, available at www.promega.com/tbs/tm313/tm313.html

Protocol

- Dilute enzyme, substrate, ATP and inhibitors in Tyrosine Kinase Buffer.
- Add to the wells of 384 low volume plate:
1 μ l of inhibitor or (5% DMSO)
2 μ l of enzyme (defined from table 1)
2 μ l of substrate/ATP mix
- Incubate at room temperature for 120 minutes.
- Add 5 μ l of ADP-Glo™ Reagent
- Incubate at room temperature for 40 minutes.
- Add 10 μ l of Kinase Detection Reagent
- Incubate at room temperature for 30 minutes.
- Record luminescence (Integration time 0.5-1second).

Table 1. ACK 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.

ACK, ng	200	100	50	25	13	6.3	3.1	1.6	0
Luminescence	231719	107967	48671	23730	12749	7352	4229	2668	671
S/B	345	161	73	35	19	11	6	4	1
% Conversion	92	42	19	9	4	2	1	0.4	0

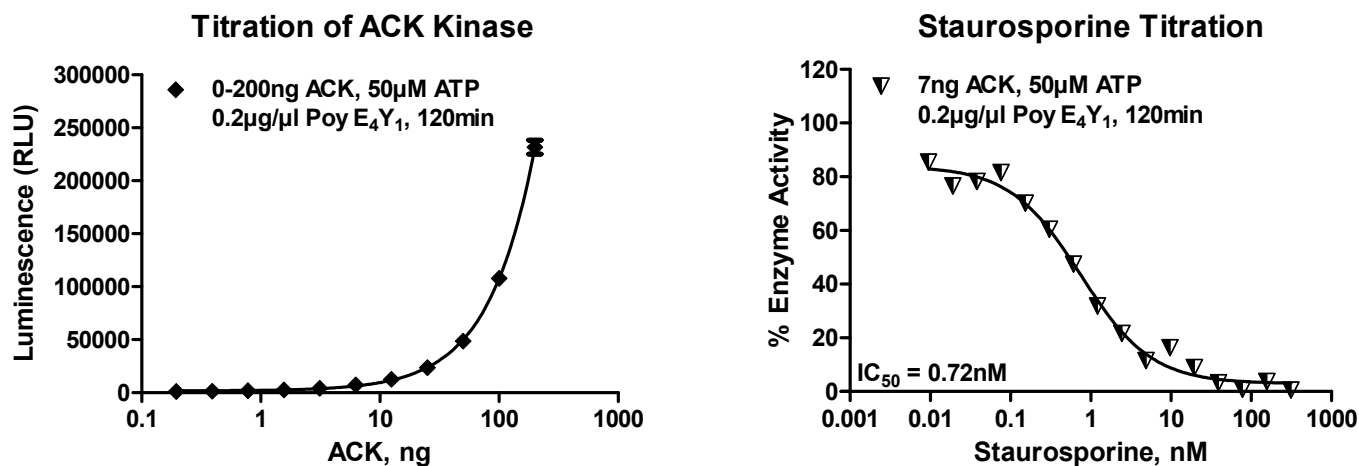


Figure 3. ACK Kinase Assay Development. (A) ACK enzyme was titrated using 50 μ M ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) Staurosporine dose response was created using 7ng of ACK to determine the potency of the inhibitor (IC₅₀).

Assay Components and Ordering Information:		Promega	SignalChem Specialists in Signaling Proteins
Products	Company	Cat.#	
ADP-Glo™ Kinase Assay	Promega	V9101	
ACK Kinase Enzyme System	Promega	V4050	
ADP-Glo™ + ACK Kinase Enzyme System	Promega	V4051	

ACK Kinase Buffer: 40mM Tris,7.5; 20mM MgCl₂; 0.1mg/ml BSA; 2.5mM MnCl₂; 50 μ M DTT.