p38γ Kinase Assay

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Scientific Background:

P38γ is a member of the p38 MAPK family which is activated in response to stress (1). p38γ gene was mapped to 22q13.3 and functions as a signal transducer during differentiation of myoblasts to myotubes. Enforced localization of p38γ in the nucleus or cytoplasm markedly attenuates the ability of the kinase to induce cell cycle arrest in fibroblasts. p38γ increases basal glucose uptake and decreases DNP- and contraction-stimulated glucose uptake, partially by affecting levels of glucose transporter expression in skeletal muscle (2).

1. Li, Z. et al: The primary structure of p38-gamma: a new member of p38 group of MAP kinases. Biochem. Biophys. Res. Commun. 228: 334-340, 1996.

2. Ho, RC. et al: p38gamma MAPK regulation of glucose transporter expression and glucose uptake in L6 myotubes and mouse skeletal muscle. Am J Physiol Regul Integr Comp Physiol. 2004 Feb; 286(2):R342-9. Epub 2003 Oct 30.

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.

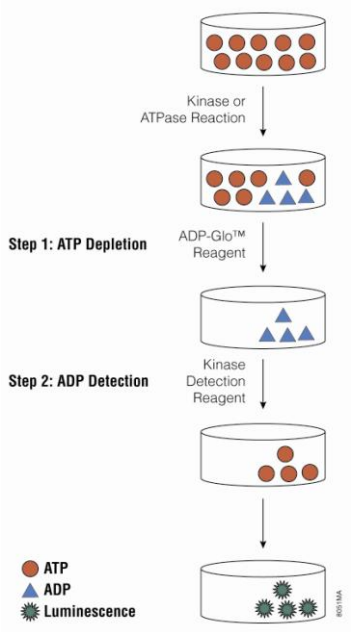

Figure 1. Principle of the ADP-Glo™ Kinase Assay. The ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.

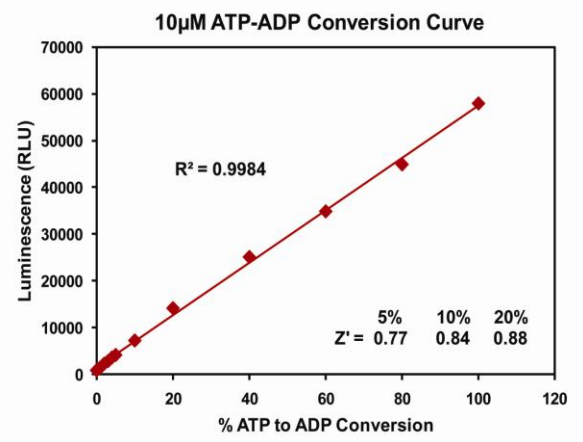

Figure 2. Linearity of the ADP-Glo Kinase Assay. ATP-to-ADP conversion curve was prepared at 10µM ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction. Z' factors were determined using 200 replicates of each of the % conversions shown.

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 Kinase 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 60 minutes.
- 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 0.5-1second).

Table 1. p38γ 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.

P38γ, ng	200	100	50	25	12.5	6.3	3.1	1.6	0.8	0.4	0.2	0
RLU	87182	82177	75414	76909	74868	60677	39888	25698	12921	7333	3960	836
S/B	104	98	90	92	90	73	48	31	15	9	5	1
% Conversion	100	95	87	89	87	70	46	29	14	7	3	0

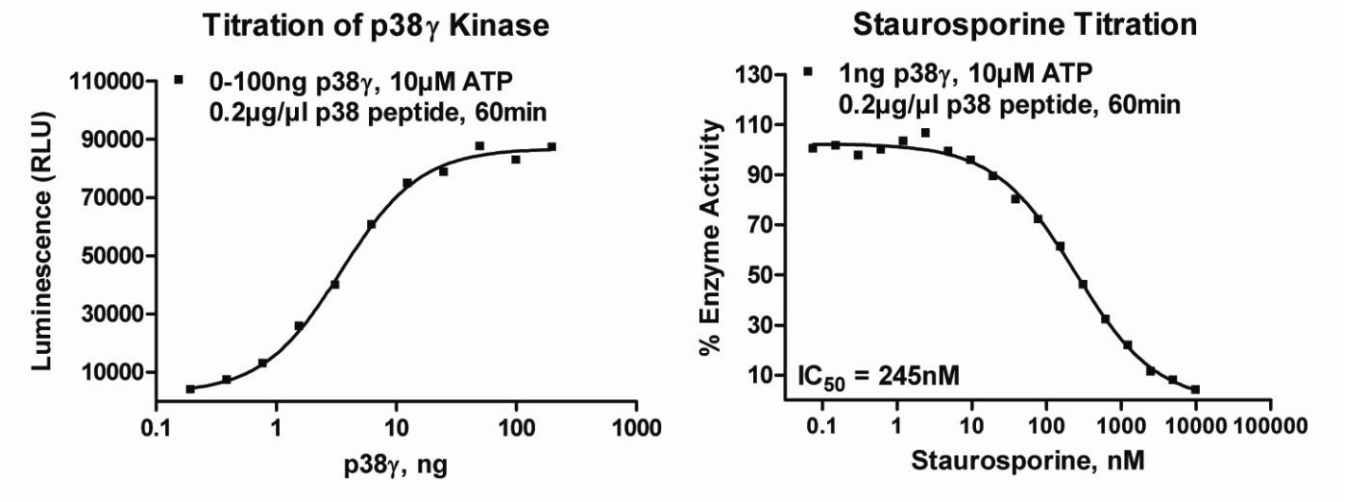

Figure 3. p38γ Kinase Assay Development. (A) p38γ enzyme was titrated using 10µM ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) Staurosporine dose response was created using 1ng of p38γ to determine the potency of the inhibitor (IC_{50}).

Assay Components and Ordering Information:

Products

	Company	Cat.#
ADP-Glo™ Kinase Assay	Promega	V9101
p38γ Kinase Enzyme System	Promega	V3371
ADP-Glo™ + p38γ Kinase Enzyme System	Promega	V9601

p38γ Kinase Buffer: 40mM Tris,7.5; 20mM $MgCl_2$; 0.1mg/ml BSA; 50µM DTT.