

MELK Kinase Assay

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Scientific Background:

MELK or maternal embryonic leucine zipper kinase is a member of the CAMKL kinase family. MELK is a key regulator of the proliferation of malignant brain tumors including their stem cells (1). MELK transcript abundance correlates with malignancy grade in human astrocytomas and represents a therapeutic target for the management of the most frequent brain tumors in adult and children. MELK also plays a role in mammary carcinogenesis through inhibition of the pro-apoptotic function of Bcl-GL (2). Therefore, the kinase activity of MELK could be a promising molecular target for development of therapy for patients with breast cancers.

1. Nakano I. et.al: Maternal embryonic leucine zipper kinase (MELK) regulates multipotent neural progenitor proliferation. *J Cell Biol* 2005; 170: 413–27.
2. Lin ML. et.al: Involvement of maternal embryonic leucine zipper kinase (MELK) in mammary carcinogenesis through interaction with Bcl-G, a pro-apoptotic member of the Bcl-2 family. *Breast Cancer Res* 2007; 9: R17.

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.

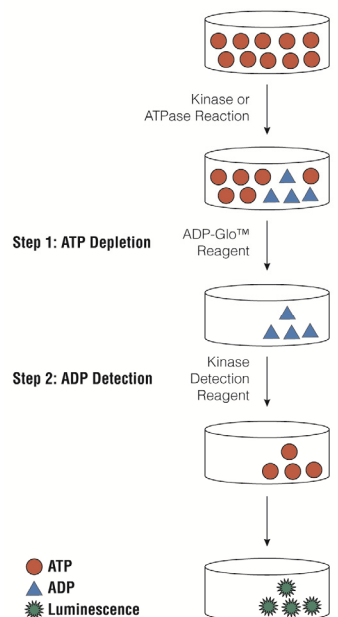


Figure 1. Principle of the ADP-Glo™ Kinase Assay. The ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.

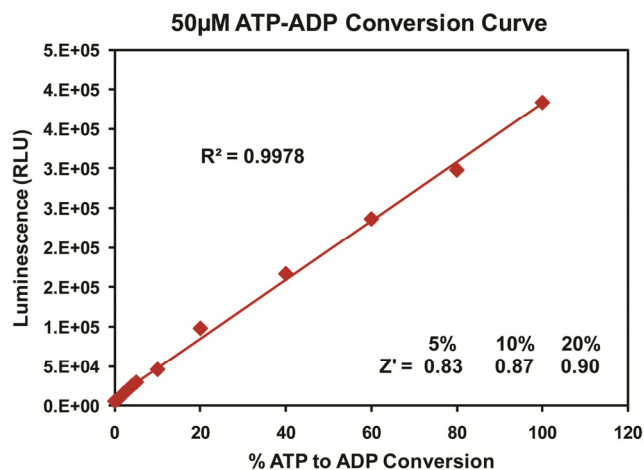
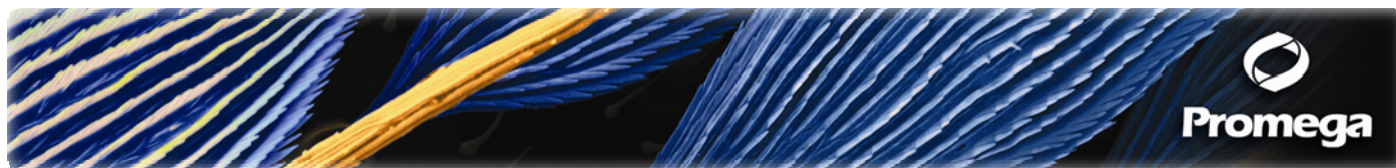


Figure 2. Linearity of the ADP-Glo Kinase Assay. ATP-to-ADP conversion curve was prepared at 50µM ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction. Z' factors were determined using 200 replicates of each of the % conversions shown.



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 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 60 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. MELK 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.

MELK, ng	200	100	50	25	12.5	6.3	3.1	1.6	0
RLU	328192	282648	241686	168324	74062	9199	5790	1447	1194
S/B	275	237	202	141	62	8	4.8	1.2	1
% Conversion	90	78	66	46	20	2	1.2	0.0	0

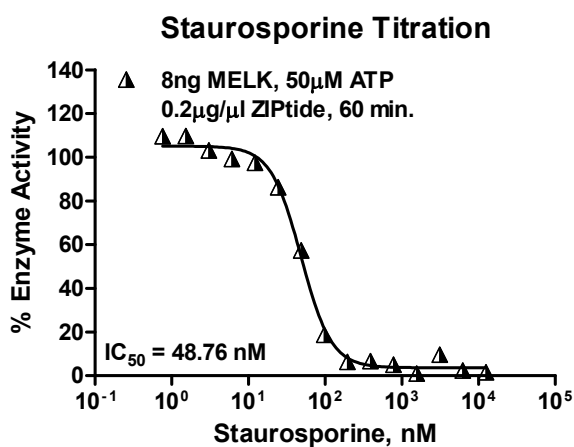
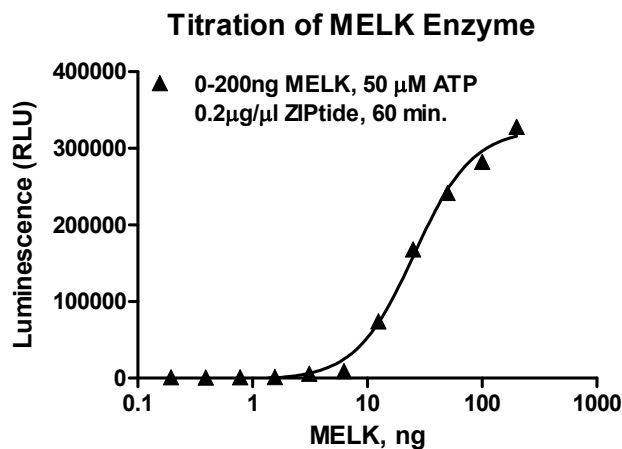


Figure 3. MELK Kinase Assay Development. (A) MELK enzyme was titrated using 50 μ M ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) Staurosporine dose response was created using 8ng of MELK to determine the potency of the inhibitor (IC_{50}).

Assay Components and Ordering Information:		Promega	SignalChem Specialists in Signaling Proteins
Products	Company	Cat.#	
ADP-Glo™ Kinase Assay	Promega	V9101	
MELK Kinase Enzyme System	Promega	V4150	
ADP-Glo™ + MELK Kinase Enzyme System	Promega	V4151	

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