

STK3 Kinase Assay

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Scientific Background:

STK3, also known as MST2, encodes a protein of 491-amino acid which contains an N-terminal catalytic domain characteristic of STKs (1). STK3 and STK4 share 94% amino acid identity in the catalytic domain and 78% identity overall. RAF1 has been shown to counteract apoptosis by suppressing the activation of mammalian sterile 20-like kinase (MST2). STK3 is a kinase that is activated by the proapoptotic agents, staurosporine and FAS ligand (2). STK3 activation presumably allows cells to resist unfavorable environmental conditions.

1. Creasy, C L. et al: Cloning and characterization of a member of the MST subfamily of Ste20-like kinases. *Gene* 167: 303-306, 1995.
2. Lee, K K. et al: MST, a physiological caspase substrate, highly sensitizes apoptosis both upstream and downstream of caspase activation. *J. Biol. Chem.* 276: 19276-19285, 2001.

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.

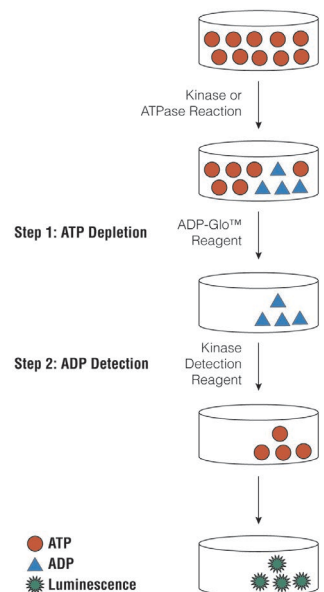


Figure 1. Principle of the ADP-Glo™ Kinase Assay. The ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.

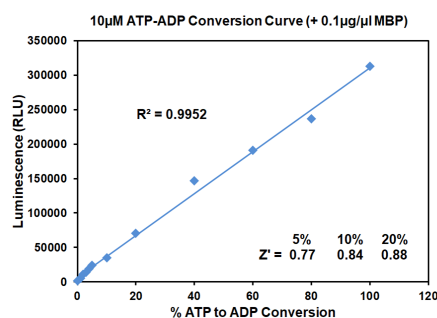


Figure 2. Linearity of the ADP-Glo Kinase Assay. ATP-to-ADP conversion curve was prepared at 10µM ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction. Z' factors were determined using 200 replicates of each of the % conversions shown.

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 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 indicated time (See Figure 3).
- 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 0.5-1 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	200	100	50	25	12.50	6.25	3.13	0.78	0
Luminescence	131,774	77,741	62,953	44,627	27,429	15,684	8,492	2,775	1,310
S/B	101	59	48	34	21	12	6	2	1
% Conversion	41	23	19	13	7	3	1	0	0

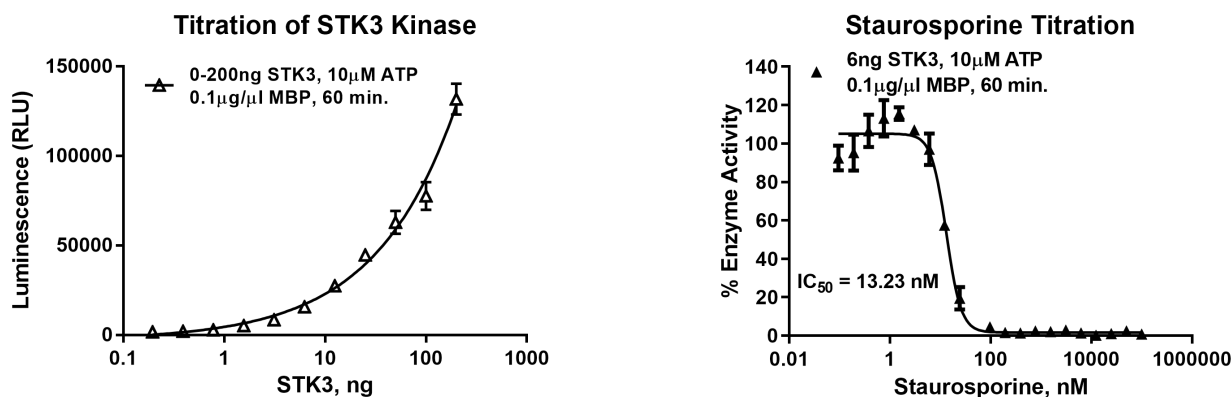


Figure 3. STK3 Kinase Assay Development. (A) STK3 enzyme was titrated using 10 μ M ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) Inhibitor dose response was created using 6ng of STK3 to determine the potency of the inhibitor (IC₅₀).



Ordering Information:

Products	Size	Cat. #
STK3 Kinase Enzyme System	10 μ g	VA7564
	1mg	VA7565
ADP-Glo™ + STK3 Kinase Enzyme System	1 Each	VA7566