GSK3α Kinase Assay

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Scientific Background:

GSK3α is a multifunctional protein serine kinase, homologous to Drosophila 'shaggy' (zeste-white3) and implicated in the control of several regulatory proteins including glycogen synthase and transcription factors (e.g., JUN) (1). GSK3α also plays a role in the WNT and PI3K signaling pathways. Alzheimer disease is associated with increased production and aggregation of amyloid-beta-40 and -42 peptides into plaques. GSK3α is required for maximal production of the beta-amyloid-40 and -42 peptides generated from the amyloid precursor protein by presenilin-dependent gamma-secretase cleavage. In vitro, lithium, a GSK3α?inhibitor, blocks the production of the beta-amyloid peptides by interfering with the gamma-secretase step (2).

1. Ali, A. et al: Glycogen synthase kinase-3 : properties, functions, and regulation. Chem. Rev. 101: 2527-2540, 2001.
2. Phiel, C J. et al: GSK-3-alpha regulates production of Alzheimer's disease amyloid-beta peptides. Nature 423: 435-439, 2003.

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.

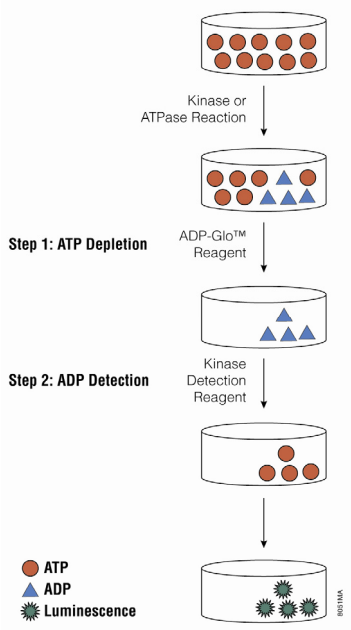

Figure 1. Principle of the ADP-Glo™ Kinase Assay. The ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.

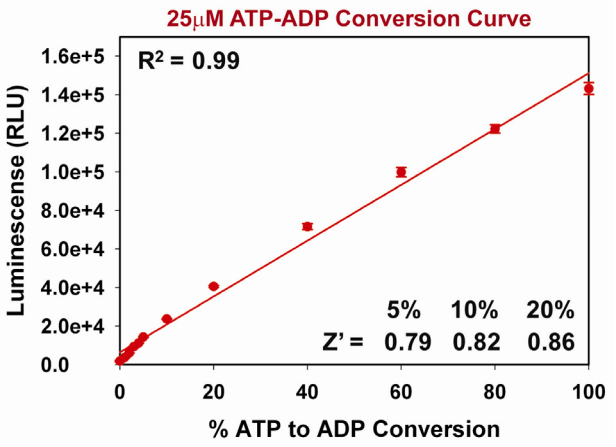

Figure 2. Linearity of the ADP-Glo Kinase Assay. ATP-to-ADP conversion curve was prepared at 25μM ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction. Z' factors were determined using 200 replicates of each of the % conversions shown.

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. GSK3α Enzyme Titration. **Reactions were carried out for 60 minutes and kinase activity was determined using ADP-Glo. 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.**

GSK3α, ng	25	12.5	6.25	3.12	1.56	0.78	0.39	0.2	0
RLU	79750	73523	58089	37532	21625	10892	5850	3331	1023
S/B	77.96	71.87	56.78	36.69	21.14	10.65	5.72	3.26	1
% Conversion	39.38	36.18	28.27	17.73	9.57	4.07	1.48	0.19	0

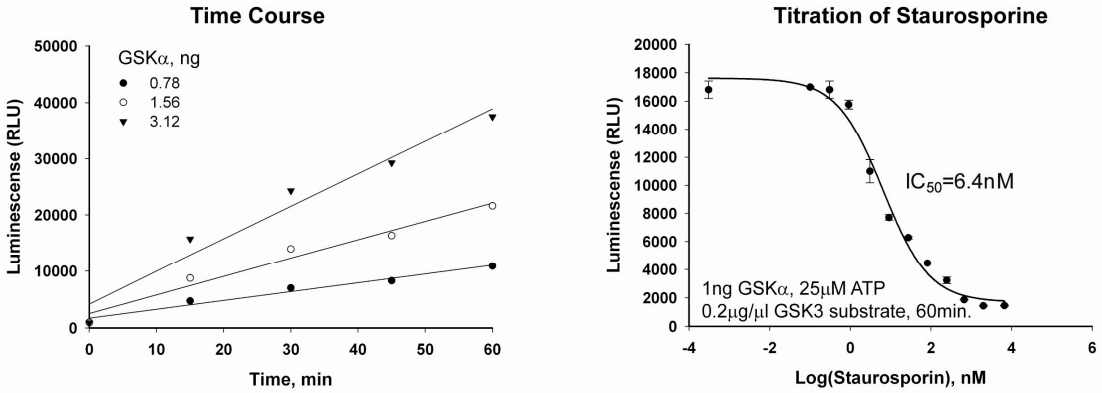

Figure 3. GSK3α Kinase Assay Development. **GSK3α linear response curves were obtained at indicated amounts of enzyme using 0.2µg/µl of GSK peptide substrate and 25µM ATP. To determine the potency of the inhibitor (IC_{50}) staurosporine dose response was performed under conditions indicated in the figure.**

Assay Components and Ordering Information:

Products	Company	Cat.#
ADP-Glo™ Kinase Assay	Promega	V9101
GSK3α Kinase Enzyme System	Promega	V3051
ADP-Glo + GSK3α Kinase Enzyme System	Promega	V9361

GSK3α Kinase Buffer: 40mM Tris, 7.5; 20mM $MgCl_2$; 0.1mg/ml BSA; 50µM DTT