# GSK3β Kinase Assay

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## Scientific Background:

**GSK3β is a serine threonine protein kinase that was originally identified as the kinase that phosphorylate and inhibits glycogen synthase (1). GSK3β is ubiquitously present in human tissues and implicated in the regulation of several physiological processes, including the control of glycogen and protein synthesis by insulin, modulation of the transcription factors AP-1 and CREB. Transient transfection of human GSK3 beta into Chinese hamster ovary cells stably transfected with individual human tau isoforms leads to hyperphosphorylation of tau at all the sites investigated with phosphorylation-dependent anti-tau antibodies(2).**

1. Sutherland C. et al: Inactivation of glycogen synthase kinase-3 beta by phosphorylation: new kinase connections in insulin and growth-factor signalling. Biochem J. 1993 Nov 15;296 ( Pt 1):15-9.
2. Sperber BR, Leight S, Goedert M, Lee VM. Glycogen synthase kinase-3 beta phosphorylates tau protein at multiple sites in intact cells. Neurosci Lett. 1995 Sep 8;197(2):149-53.

## 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.**

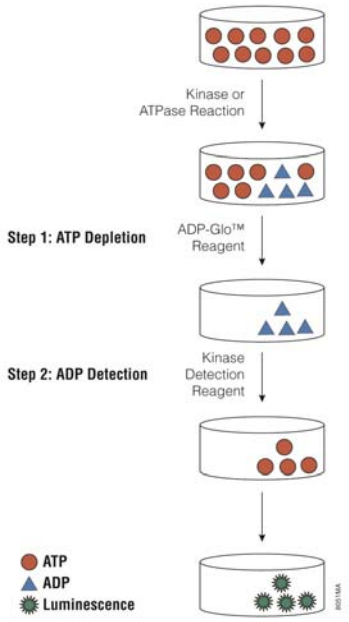


**Figure 1. Principle of the ADP-Glo™ Kinase Assay.** The ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.

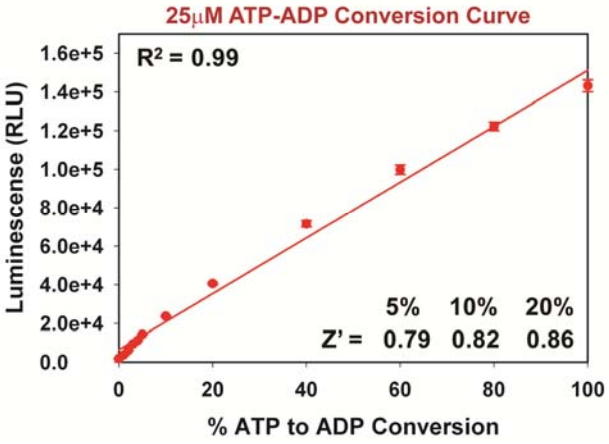


**Figure 2. Linearity of the ADP-Glo Kinase Assay.** ATP-to-ADP conversion curve was prepared at 25μM ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction. Z' factors were determined using 200 replicates of each of the % conversions shown.

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. GSK3β 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.**

| GSK3β, ng | 25 | 12.5 | 6.25 | 3.12 | 1.56 | 0.78 | 0.39 | 0.2 | 0 |
|---|---|---|---|---|---|---|---|---|---|
| RLU | 77739 | 72874 | 55009 | 34627 | 18980 | 9522 | 5257 | 3386 | 1050 |
| S/B | 73.97 | 69.34 | 52.34 | 32.95 | 18.06 | 9.06 | 5.00 | 3.22 | 1 |
| % Conversion | 82.52 | 77.21 | 57.70 | 35.45 | 18.37 | 8.04 | 3.39 | 1.34 | 0 |

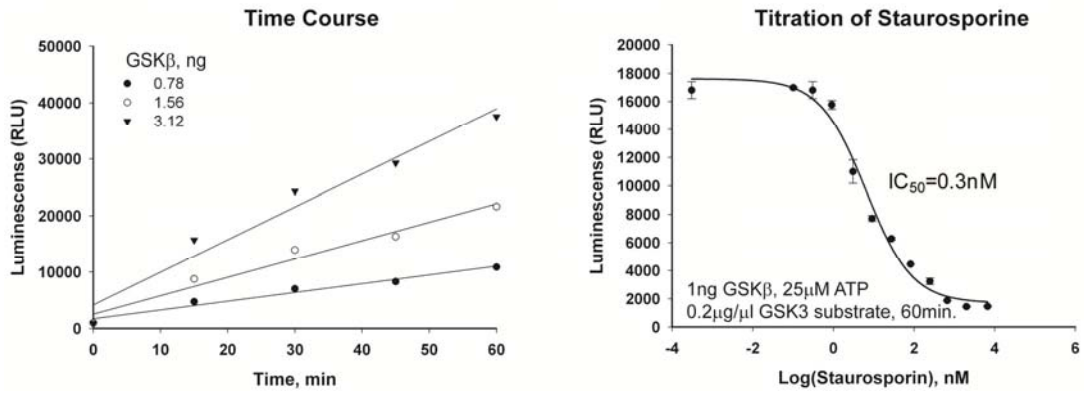


**Figure3. GSK3β Kinase Assay Development.** **GSKβ linear response curves were obtained at indicated amounts of enzyme using 0.2µg/µl of GSK peptide substrate and 25µM ATP. To determine the potency of the inhibitor ($IC_{50}$) staurosporine dose response was performed under conditions indicated in the figure.**

**Assay Components and Ordering Information:**

| Products | Company | Cat.# |
|---|---|---|
| ADP-Glo™ Kinase Assay | Promega | V9101 |
| GSK3β Kinase Enzyme System | Promega | V1991 |
| ADP-Glo + GSK3β Kinase Enzyme System | Promega | V9371 |

GSK3β Kinase Buffer: 40mM Tris, 7.5; 20mM $MgCl_2$; 0.1mg/ml BSA; 50µM DTT

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