

BTK (C481S) Kinase Assay

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Scientific Background:

BTK (also known as Bruton tyrosine kinase) plays a crucial role in B-lymphocyte differentiation and activation. BTK interacts with SRC homology 3 domains of FYN, LYN and HCK that are activated upon stimulation of B- and T-cell receptors (1). Defects in the BTK gene cause Agammaglobulinemia, an X-linked immunodeficiency characterized by failure to produce mature B lymphocyte cells and associated with a failure of Ig heavy chain rearrangement. The unique role of BTK makes it a desirable target for potential anti-cancer, anti-inflammatory and anti-viral agents as well as other treatments (2).

1. Cheng, G. et al: Binding of Bruton's tyrosine kinase to Fyn, Lyn, or Hck through a Src homology 3 domain-mediated interaction. Proc. Nat. Acad. Sci. 91: 8152-8155, 1994.
2. Vassilev, A O. et al: Therapeutic potential of inhibiting Bruton's tyrosine kinase, (BTK). Curr Pharm Des. 2004;10(15):1757-66.

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.

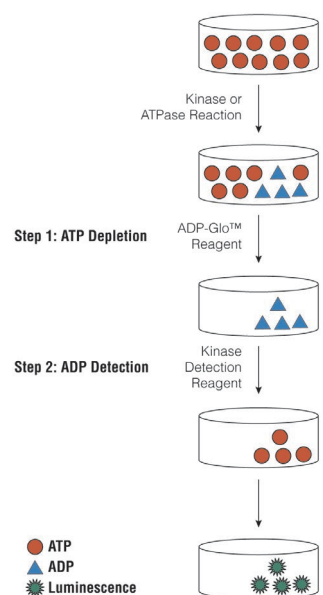


Figure 1. Principle of the ADP-Glo™ Kinase Assay. The ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.

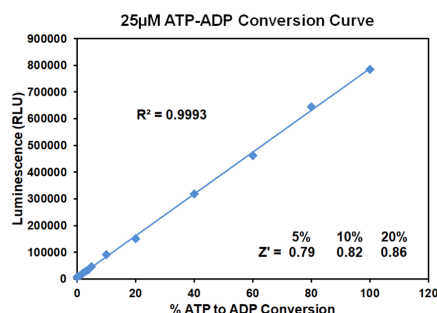


Figure 2. Linearity of the ADP-Glo Kinase Assay. ATP-to-ADP conversion curve was prepared at 25µM ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction. Z' factors were determined using 200 replicates of each of the % conversions shown.

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	180	90	45	22.50	11.25	5.63	2.81	1.41	0.70	0
Luminescence	1,010,373	646,183	449,740	250,054	135,147	61,797	32,594	14,473	7,346	4,610
S/B	219	140	98	54	29	13	7	3	2	1
% Conversion	126	80	56	31	17	7	4	1	1	0

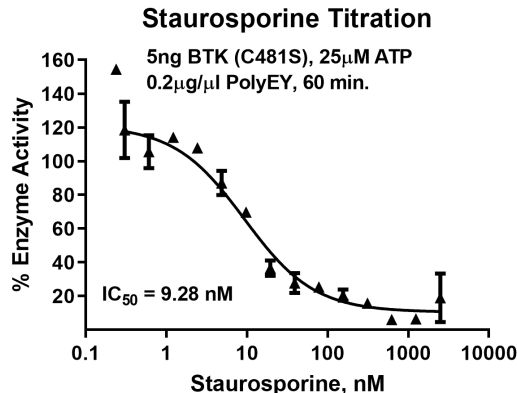
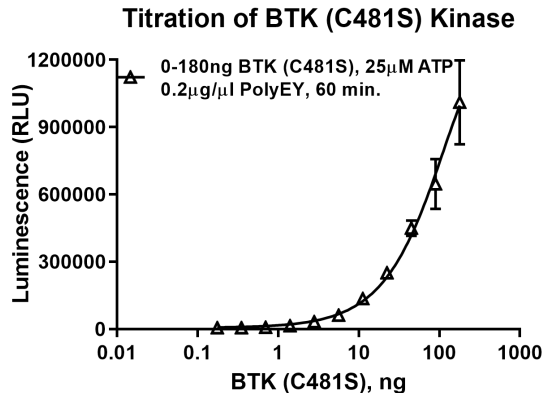


Figure 3. BTK (C481S) Kinase Assay Development. (A) BTK (C481S) enzyme was titrated using 25 μ M ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) Inhibitor dose response was created using 5ng of BTK (C481S) to determine the potency of the inhibitor (IC_{50}).



Ordering Information:

Products	Size	Cat. #
BTK (C481S) Kinase Enzyme System	10 μ g	VA7033
	1mg	VA7034
ADP-Glo™ + BTK (C481S) Kinase Enzyme System	1 Each	VA7035