ADP-Glo™: A luminescent ADP detection Assay for Kinases, and Other ADP-generating enzymes

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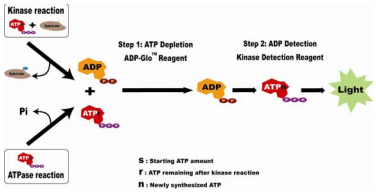
1. Abstract

Because of the increasing recognition of kinases as a validated drug targets, there have been an intense research interest in the development of technologies that monitor the activity of these enzymes. Although several technologies were developed, most suffer from a variety of limitations that makes it difficult to address all the needs of kinase screening and profiling with one platform in an attempt to develop novel therapeutics. Towards this goal we have developed ADP-Glo™, a homogenous luminescence-based ADP detection assay that is applicable to all types of kinases, ATPases and other ADP producing enzymes. The ADP-Glo™ assay is universal, applicable to all kinds of kinase substrates regardless of their nature with no prior modification (peptides, proteins, alcohols, lipids, and sugars). Instead of monitoring ATP depletion (Kinase Glo®), ADP-Glo™ is a positive response assay that monitors ADP production. It detects ADP at early stages of enzyme reactions with very high signal to background (SB) ratio. It can be performed at wide range of ATP concentrations (low micro molar to millimolar), detects low ADP concentrations, and it can be carried out in high density plate formats. The assay is robust as is indicated by the high Z' values (over 0.7) and does not require antibodies or custom synthesized substrates. Because of the high dynamic range and more importantly, the high SB values at low % ATP to ADP conversion, the assay allows the use of less amount of enzyme during high-throughput screenings. Finally, as ADP-Glo™ Assay can be used at cellular levels of ATP (mM), it is now possible to study in vitro, the mode of action of kinase inhibitors (e.g. ATP competitive vs. non competitive inhibitors) and the mechanisms of drug resistance of mutated kinases.

2. ADP-Glo™ is a positive detection assay for product formation

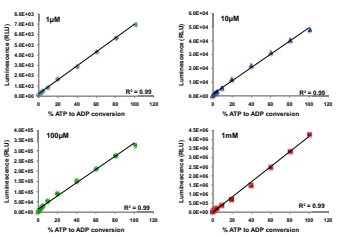


Assay format in 384 well plate
5µl kinase reaction
+
5µl ADP-Glo™ reagent
40 min. Incubation
+
10µl Kinase Detection Reagent
30-60 min. Incubation
Read Luminescennce

- Step 1: Depletion of unconsumed ATP after the kinase/ATPase reaction
- Step 2: ADP is converted into ATP that is detected via a luciferase/luciferin reaction
- Luminescent signal is proportional to ADP produced and the kinase/ATPase activity.

3. ADP-Glo™ Assay can be used with a broad range of ATP concentrations

ADP standard curves at different ADP/ATP concentrations



% ATP to ADP Conversion

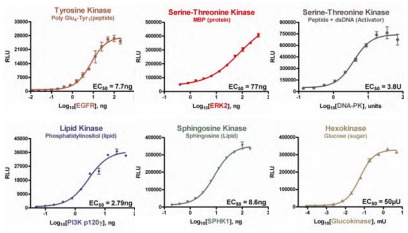
ATP-ADP Series	100	80	60	40	20	10	5	4	3	2	1	0
1µM	37	30	23	16	9	6	3.2	2.8	2.2	2.3	1.4	1.0
10µM	93	76	61	43	24	12	7	5.7	4.7	4.5	2.1	1.0
100µM	102	86	68	48	29	16	10	8	7	6	3	1
1mM	127	99	74	45	22	12	8	6	6	4	2	1

Signal to Background ratios

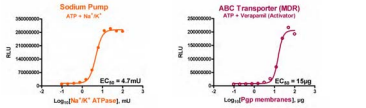
- Sensitivity: ADP-Glo™ detects as low as 20nM ADP in 10µl (0.2pmole)
- Linearity: ADP-Glo™ detects up to 1mM ADP in a linear fashion

4. ADP-Glo™ is a universal assay for Kinases and ATPases

Diverse Kinase-Substrate combinations



Beyond Kinases: ATPases



- ADP-Glo™ detects the activity of any ADP producing enzyme

5. ADP-Glo™ is a luminescent assay with high dynamic range

SB5: Amount of Enzyme to give a Signal to Background ratio of 5

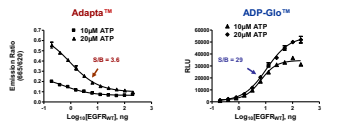
Enzyme	SB5	% ATP to ADP* conversion at SB5
EGFR	0.3ng	2.2
PDGFRα	21ng	4.7
VEGFR2 (KDR)	1ng	4.2
DNA-PK	0.4ng	2.7
AKT2	7ng	4.3
IKKβ	4.5ng	2.9
MAPK (ERK2)	1ng	5.6
PKA	0.013 units	0.35
Hexokinase	0.1 units	4.6
PI3 Kinase γ	0.15ng	2
Sphingosine Kinase 1	0.8ng	2.9
MDR1 (P-gp)	25µg (SB20)	50
Na+/K+ ATPase	1ng	3

* Enzyme reactions were performed at different ATP Concentrations.

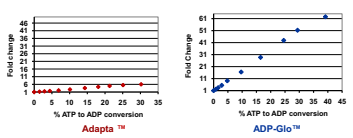
- ADP-Glo™ produces high signal to background (SB) with all enzymes tested.
- To generate a Signal to Background of 5, only a small amount of enzyme is needed when using ADP-Glo™ Assay.

6. ADP-Glo™ Assay sensitivity compared to Adapta™ assay

EGFR kinase titration with Adapta ™ compared to ADP-Glo™



Fold change generated by ADP-Glo™ and Adapta™ assays at different percentage of ADP produced during EGFR kinase reaction



- ADP-Glo™ produces a positive response and high fold change with increasing amounts of ADP.
- Adapta™ assay from Invitrogen is a negative response assay with very low signal to background at all ADP concentrations produced.

7. Comparing ADP-Glo™ to Adapta™ in assay development

Fold change produced by ADP-Glo™ and Adapta™ assays at different amount of EGFR and IKKβ kinases

EGFR assay

Enzyme (ng)	ADP-Glo	Adapta
100	69.5	6
50	62.5	5.5
25	51	5
12.5	43	4.5
6.3	29	3.6
3.1	16.5	3
1.6	9	2
0.8	5.5	2
0.4	3.5	1.5
0.2	2	1.2
0	1	1

Adapta™ SB5.5 50ng

ADP-Glo™ SB5.5 0.8ng

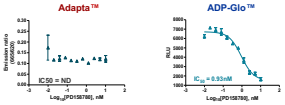
IKKβ assay

Enzyme (ng)	ADP-Glo	Adapta
200	48	4.5
120	49.5	4
72	44	3.5
43	36	3
26	25	2.5
15.6	11.5	2
9.3	9	1.5
5.6	6	1.3
3.4	4.5	1.2
2	2.5	1.1
0	1	1

Adapta™ SB4.5 200ng

ADP-Glo™ SB4.5 3.4ng

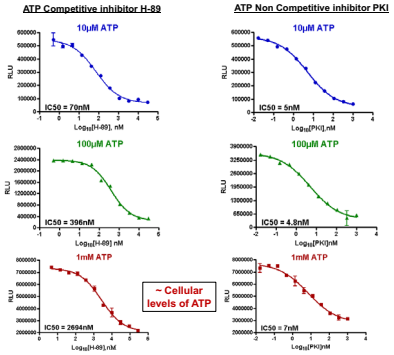
Titration of EGFR inhibitor PD158780 using enzyme amounts corresponding to SB 10 (1.77ng)



- ADP-Glo™ requires less amount of enzyme to successfully screen for inhibitors (40 to 60 times less).

8. Determination of inhibitor's mechanism of action using ADP-Glo™

Determining IC_{50} for ATP competitive and non competitive inhibitors of PKA



- ADP-Glo™ Assay can be used at cellular levels of ATP (mM).
- ADP-Glo™ is a perfect assay to distinguish between ATP competitive and non competitive kinase inhibitors.

9. Features to remember about the ADP-Glo™ Assay

- Homogenous, non radioactive and Antibody Free
- Luminescent assay: Less Compound interference
- Positive Response: Signal is proportional to ADP produced
- Stable luminescent signal: Batch plate processing
- Universal: Any kinase-substrate combination and ATPases
- Robust Assay (Z' higher than 0.7)
- High dynamic range: High Signal to Background at low % ATP to ADP conversion allows use of lower amount of enzyme during HTS (Lowering the cost)
- Broad range of ATP conc. (linear from µM to mM range) allows distinction between ATP competitive and Non-competitive inhibitors
- High sensitivity: 20nM ADP detected with more than 2.5 fold difference