Automation of ADP-Glo™ Universal Kinase Assay For HTS Screening AND Profiling Applications

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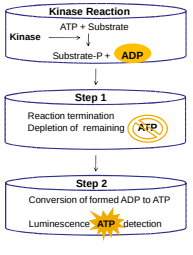
1. Abstract

Growing recognition of kinases as a cornerstone in cell signaling and their increasing importance as drug targets has generated a need for developing technologies that enable rapid characterization of more than 500 kinases present in genome. Successful adaptation of such technologies on automated liquid handling platforms is absolute necessity for generating large amount of data required for kinases characterization and their inhibitors evaluation.

Here using Promega's recently introduced universal ADP-Glo™ kinase assay we show how combining integrated instrumentation allows researchers efficiently screen and profile compounds for potential identification of new drugs. For this work we have used: automated liquid handling platforms - Beckman Coulter BioRAPTR™ or Tecan TeMo™ workstations with multifunctional plate readers - BMG PHERAstar™ or Tecan Safire2™ or Infinite500™.

ADP-Glo™ assay monitors ADP formation, the common product generated in all kinases reactions providing universal platform for all kinases types characterization. Even though the ADP-Glo™ assay requires coupling addition liquid handling steps with wide volume range, as we show here, the combination of advanced chemistry and robust performance on a variety of automated workstations makes the ADP-Glo™ assay attractive for HTS applications.

2. Principal of ADP-Glo™: Luminescence ADP detection assay



- Positive output: Detects product formation
- Luminescent: extremely low background, high sensitivity and low false hits
- One format is suitable for diverse groups of substrates: peptides, proteins, lipids, sugars
- High signal to background at low ATP/ADP conversion rates
- Broad linear range of ATP (μM to mM)

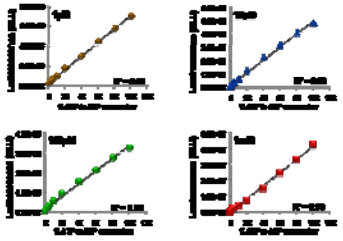
3. Can be automated for low volume HTS screening applications

Optimization of automated ADP-Glo™ assay.
ADP-Glo™ assay can be adopted to non-contact or contact dispensers.
As non-contact dispenser Beckman Coulter BioRAPTR™ workstation was used. It is designed to dispense 100nl to 60ul volumes in to 384, 1536 and 3456 well plates.
As contact dispenser Tecan TeMo™ liquid handling system was used, it is designed to dispense 2 - 200ul in to up to 384 well plates.
For Luminescence detection multifunctional plate readers where used: BMG PHERAstar™ or Tecan Safire2™ for 384 and 1536 well plates and Tecan Infinite500™ for 384 well plates.
Experiments where performed in 1536 and Low Volume 384 well plates.

	Volume, µl / well	
	Plate Format	
Reagents Dispensed:	384	1536
Staurosporine	1	0.5
cAMP - Dependent Protein Kinase Catalytic Subunit	2	1
Kemptide + ATP	2	1
ADP-Glo™ Reagent (Cat.# V912B)	5	2.5
Kinase Detection Reagent (Cat.# V913B and V914B)	10	5

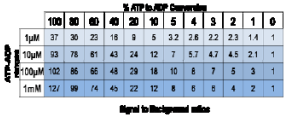
4. HTS screens can be performed at wide ATP ranges

Wide dynamic range with linear response



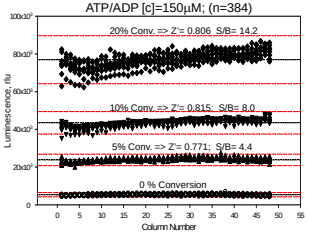
High sensitivity

- Detects as low as 20nM ADP
- Detects 5% of product phosphorylation with signal to background > 5 fold



5. Shows Z' >0.7 even at low product formation rates

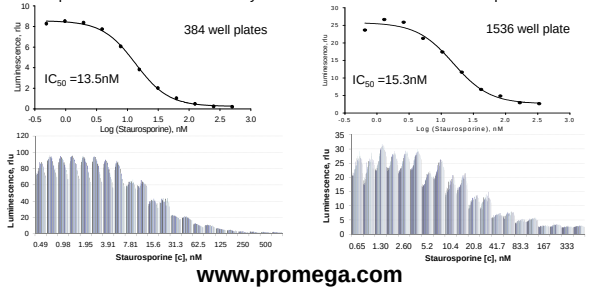
Z' values at varying ATP concentration and 5,10, 20% of ATP to ADP conversion in 384 and 1536 well plates



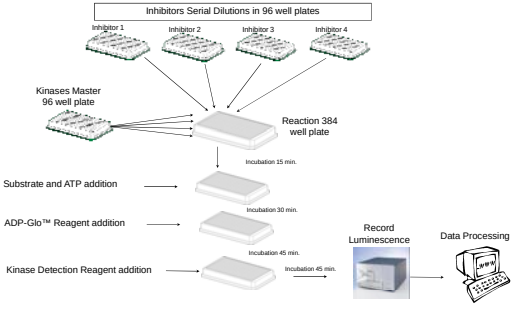
ATP, µM	% of ATP to ADP conversion					
	5		10		20	
	384	1536	384	1536	384	1536
5	0.839	0.505	0.827	0.701	0.904	0.803
10	0.768	0.634	0.845	0.742	0.883	0.802
25	0.795	0.729	0.828	0.791	0.868	0.815
50	0.830	0.718	0.872	0.790	0.903	0.807
150	0.820	0.771	0.863	0.815	0.880	0.804
250	0.761	0.713	0.828	0.793	0.775	0.757

6. Provides robust system for inhibitor screening

To evaluate robustness of ADP-Glo™ performance for inhibitor screening
48 or 96 data points for each inhibitor concentration was collected.
cAMP - Dependent Protein Kinase Catalytic Subunit was titrated with Staurosporine.

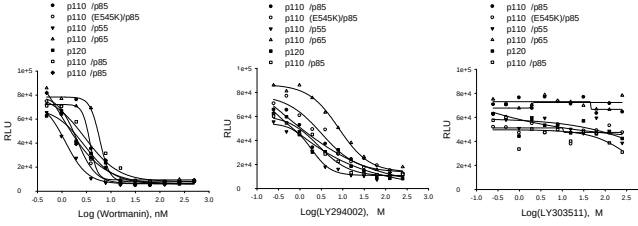


7. Provides universal platform for kinase profiling



8. Example of lipid kinase profiling

Universality of ADP-Glo™ assay allows rapid set up for kinase profiling



9. Summary

The combination of the ADP-Glo™ advanced chemistry and the robust performance on a variety of automated workstations address the need of integrated platform for small molecule screening and profiling across the entire human kinome

- The assay is scalable and retains the sensitivity in miniaturized formats (Low Volume 384 and 1536 well plates)
- Inhibitor screens can be performed at wide ATP concentrations with Z' values >0.7 even at low substrate conversion rates (5%)
- Assay universality offers flexibility and convenience for setting up profiling screens with diverse groups of kinases