Comparison of ADP Detection Methods Used for High Throughput Screening

BellBrook Labs

Robert G. Lowery, Karen M. Kleman, Thomas K. Zielinski, Andrew L. Kopp, Meera Kumar

BellBrook Labs 5500 Nobel Drive Suite 250 Madison, WI USA

Abstract

ADP detection is an attractive approach for screening kinases and other ATP-utilizing enzymes because it provides a universal platform that can be used for any member of the kinase superfamily as well as many other ATP-dependent enzymes, regardless of the acceptor substrate. The three ADP detection approaches that have been developed into commercial HTS assay products are 1) direct immunodetection of ADP, which relies on antibodies that selectively recognize ADP in the presence of excess ATP (Transcreener® ADP² Assay, BellBrook Labs), and 2) enzyme-coupled detection, where the ADP is used to drive a cascade of detection enzymes that ultimately produces a fluorescent signal (ADP Quest™/ADP Hunter™, DiscoverX), and 3) enzyme-coupled detection, where the residual ATP is first depleted and then ADP is converted to ATP and detected using luciferase (ADP Glo™, Promega). All three methods provide robust, initial velocity detection of kinases and other ATP-dependent enzymes and have been used in HTS laboratories worldwide. Here we compare these assay methods with respect to assay principle, protocol, performance, and adaptation for diverse screening and profiling applications as reflected in the scientific literature.

Transcreener is Simpler, More Sensitive and More Stable than Coupled Enzyme Assays

Feature	Transcreener ADP² Assay	ADP-Glo¹	ADP-Quest²
Reagent Additions	1	2	2
Sensitivity	1 nM¹	20 nM	600 nM
Kinetic Mode	yes	no	yes
Assay Method	Immunodetection of ADP	Coupled Enzyme Assay (3 enzymes)	Coupled Enzyme Assay (3 enzymes)
Reagent Stability	>3 weeks at RT (FP)	24 hours at RT	1 week at 2-8°C
Signal Stability	>24 hours at RT	5 hours @ RT	6 hours @ RT
Detection Temperature	Flexible	Signal is temperature dependent	Signal is temperature dependent
Detection Modes	TR-FRET, FI, FP	Luminescence	FI

Table 1. Comparison of ADP Detection Assays. Information for ¹ADP Glo and ²ADP Quest assays are from the user manuals found on the Promega and DiscoverX websites, respectively. Information for Transcreener ADP2 Assays are from the Technical Manual and from Kleman-Leyer, et al (2009) Assay and Drug Dev Tech 7: 56-67.

Robust Kinase Detection with Low ATP

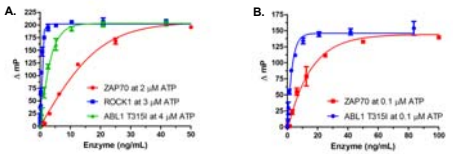

Figure 4. Detection of kinases at low ATP concentrations. A. ATP at K_m concentrations. B. 0.1 µM ATP. Kinases are often screened using the K_m concentration for ATP, but use of lower ATP concentrations is sometimes desirable to bias screens more toward competitive inhibitors and decrease enzyme usage.

Overnight Reagent and Signal Stability

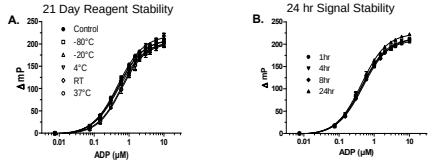

Figure 5. Overnight reagent and signal stability. Standard curves for conversion of 10uM ATP to ADP were used to measure **A. Stability of Transcreener detection reagents prior to addition to reactions and B. The stability of the signal following addition to kinase reactions.** Data is for the FP assay, the FI and TR-FRET assays also have at least overnight reagent and signal stability.

Transcreener ADP² Assays: Direct Detection of ADP with FP, TR-FRET and FI Readouts

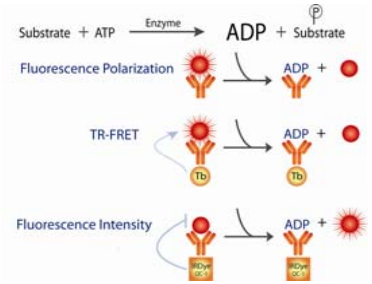

Figure 1. Transcreener assays are the only direct ADP detection method available. ADP displaces tracer from a highly specific monoclonal antibody resulting in a change in fluorescence, with fluorescence polarization (FP), time resolved FRET (TR-FRET) and fluorescence intensity (FI) formats available. All three are homogenous, mix and read assays and use a red-shifted tracer to minimize compound interference.

Coupled Enzyme Assay ADP Detection Methods

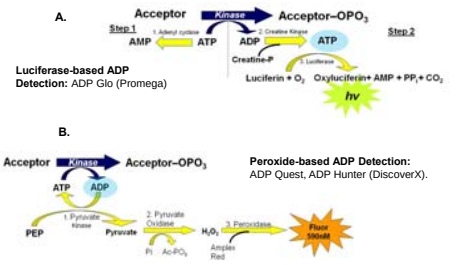

Figure 2. Coupled enzyme assay ADP detection methods. ADP detection methods other than Transcreener rely on coupling enzymes, which convert the ADP to a detectable product in a series of enzymatic steps. **A. ADP Glo™ assay (Promega)**: Residual ATP is first converted to AMP by adenyl cyclase; then ADP is converted to ATP and detected by luciferase. **B. ADP Quest™ assay (DiscoverX):** Two enzymatic steps are used to generate hydrogen peroxide, which reacts with Amplex Red to produce a fluorescent product in a third enzymatic step.

High Z' Values at Low ATP Conversion in Any Plate Reader

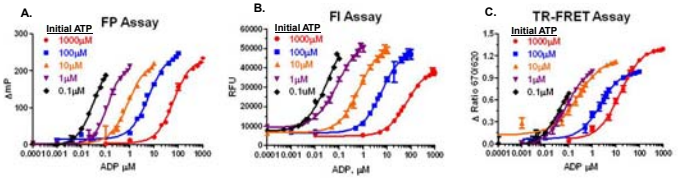

Figure 3. Standard curves for conversion of ATP to ADP demonstrate the outstanding response of the Transcreener ADP² Assays at different initial ATP concentrations. Standard curves are used to mimic enzyme reactions. Starting at the indicated concentrations of ATP, ADP is titrated and ATP is decreased proportionately. All experiments were run in in 384 well format with 24 replicates. **A. FP reactions** were read in Tecan Safire²™. **B. The TR-FRET reactions** were read in BMG Labtech's PHERAstar Plus reader. **C. The FI assay** was measured using Perkin Elmer's EnVision.

	1 µM ATP/ADP standard curve		10 µM ATP/ADP standard curve		100 µM ATP/ADP standard curve	
	Z' at 10% Conv	LLD (µM)	Z' at 10% Conv	LLD(µM)	Z' at 10% Conv	LLD(µM)
Transcreener FP	0.86	0.02 ±0.07	0.85	0.01 ±0.12	0.89	1.0 ±0.3
Transcreener TR-FRET	0.71	0.10 ±0.06	0.72	0.10 ±0.09	0.72	1.0 ±0.3
Transcreener FI	0.92	0.03 ±0.01	0.88	0.05 ±0.04	0.92	0.5 ±0.4
Luc-ADP Detection Assay	ND	0.40 ±0.87	0.30	0.50 ±0.32	0.62	5.0 ±0.7
Luc-ATP Depletion Assay	ND	0.25 ±0.40	ND	1.50 ±0.30	0.52	7.0 ±0.6

Table 2. The high sensitivity of the Transcreener ADP² Assays allows detection of 10% conversion of ATP even at low levels of ATP. Assay statistics, including Z' values and lower limits of detection (LLD) were calculated and compared with ADP Glo and Kinase Glo (Promega) reactions run under identical conditions.

Extensive Validation with Diverse Targets

Assay	# Refs	Targets	1536¹	Max Size²
Transcreener ADP² Assay	9	Var. protein kinases, Var. lipid kinases, Hsp90, Hsp72, RNA triphosphatase, Acetyl-CoA Carboxylase, RecA, Adenylosuccinate synthase, OMP decarboxylase	Yes	500,000
ADP Glo	3	Various protein kinases, PI4 Kinase	No	<1,000
ADP Quest	1	MurD ligase	No	1,000

Table 3. Independent, peer reviewed publications for ADP detection assays. ADP Glo and Transcreener references are shown on the Promega and BellBrook websites, respectively; ADP Quest references are from a PubMed search for "ADP Quest" OR "ADP Hunter" OR (ADP AND Discoverx). Publications with authors from BellBrook, Promega or DiscoverX were not included. ¹Assay was adapted to 1536 well format in at least one publication. ²Refers to the maximum size library screened in any of the publications.

Conclusions

- The Transcreener ADP² Assay is the simplest ADP detection method available, relying on direct immunodetection instead of coupled enzyme assays.
- This results in advantages over other methods with respect to sensitivity, reagent and signal stability and ease of use.
- The greater sensitivity of the Transcreener ADP² Assay allows the practical use of ATP concentrations as low as 100nM.
- The overnight reagent and signal stability of the Transcreener ADP² Assay provides flexibility for automated HTS protocols with large numbers of plates.
- The Transcreener ADP² Assay has been more extensively validated in peer reviewed publications with respect to the number and diversity of targets, the size of the screens, and adaptation to a 1536 well format.

Acknowledgements

Funding for this work was provided in part by NIH grant R44 CA110535 from the National Cancer Institute.