Transcreener-ADP Glo Comparison: Enzyme Titration

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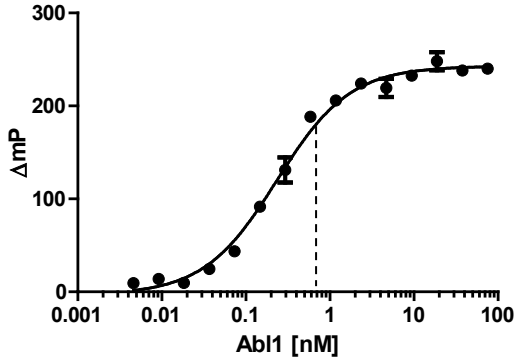
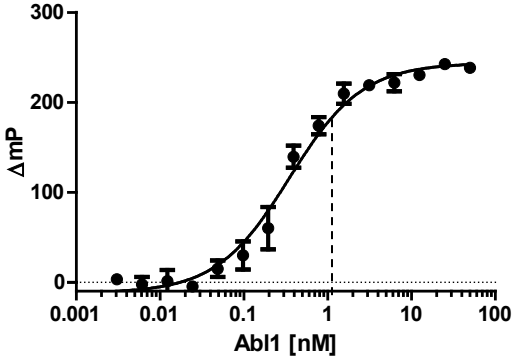
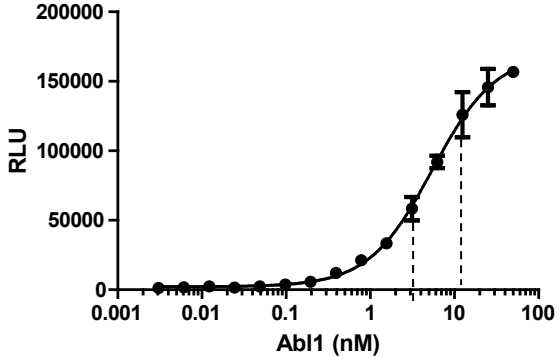
Transcreener FP - 10 µL enzyme reaction



Transcreener FP- 5 uL enzyme reaction



ADP Glo – 5 µL enzyme reaction



Between 5X and 10x <u>less</u> enzyme with Transcreener compared to ADP-GLO!

Assay	EC_{80}	S/B_{10}
TS FP, 10µl	8 fmol	
TS FP, 5µl	5 fmol	
ADP-Glo, 5µl	50 fmol	15 fmol

Why Sensitivity Matters

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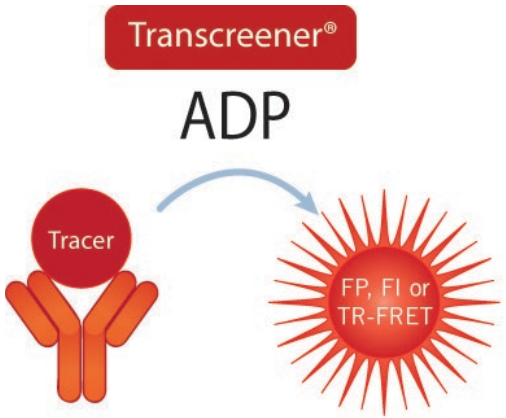
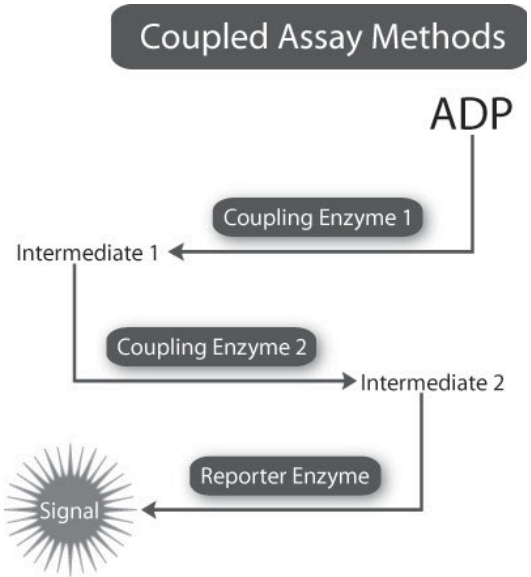
- Enzyme Cost – large screens use milligrams to grams of enzyme.
  - Less enzyme = Big Savings
- Accuracy – inhibitor accuracy can only occur when using low amounts of enzyme. 2X-3X more enzyme than IC_{50}
- Use lower amounts of substrate

Direct Detection of Nucleotides Decreases the Potential for Interference





Transcreener® assays rely on direct detection of ADP. Binding of tracer to antibody causes a change in fluorescence. There are just two components, and no intermediate steps.

All other ADP assays use indirect detection and are more complex. In a series of enzymatic steps, UDP is converted to a detectable product. Each step is subject to inhibition by library compounds.

Transcreener ADP^2 FP Assay: Overnight Reagent and Signal Stability

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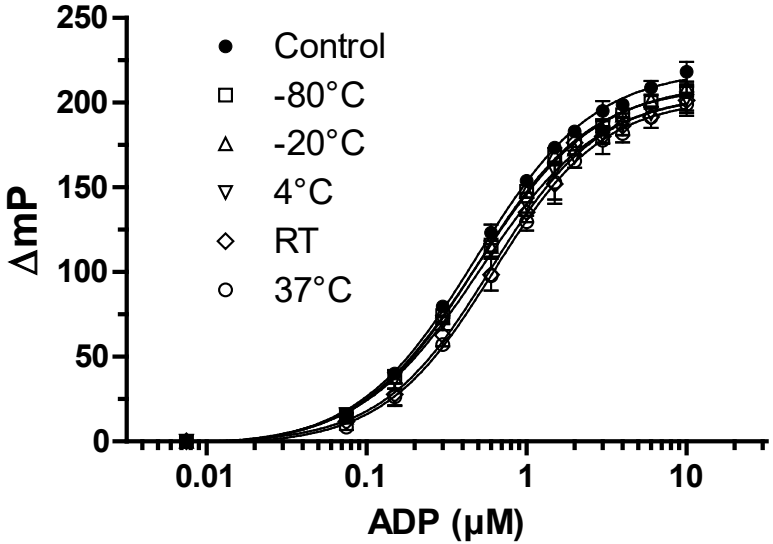
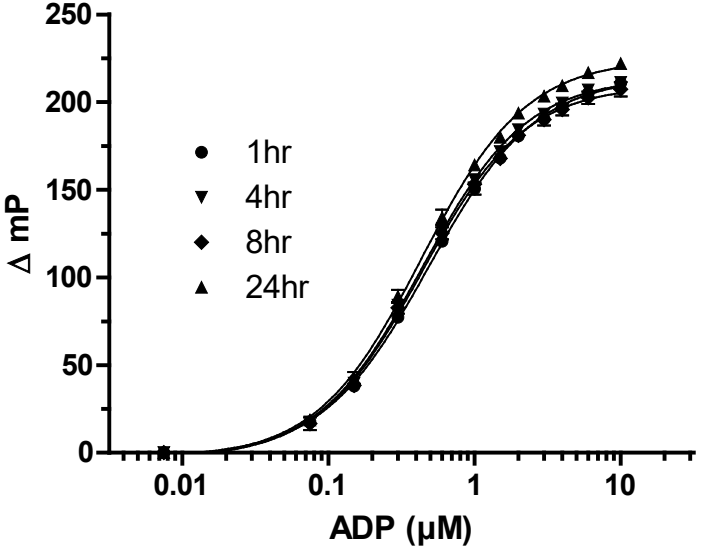
21 Day Reagent Stability



24 Hour Signal Stability



Continuous Assays

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- Run end point or continuous mode
- Allows for easier assay development
- Experimental flexibility
- Residence time determination

Features of Transcreener ADP^2 Assays

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Feature	Transcreener ADP^2 Assay	ADP-Glo^1
Reagent Additions	**1**	2
Sensitivity	**1 nM^1**	20 nM
Kinetic Mode	**yes**	no
Assay Method	**Immunodetection of ADP**	Coupled Enzyme Assay (3 enzymes)
Reagent Stability	**>3 weeks at RT (FP)**	24 hours at RT
Signal Stability	**>24 hours at RT**	5 hours @ RT
Detection Temperature	**Flexible**	Signal is temperature dependent
Detection Modes	**TR-FRET, FI, FP**	Luminescence

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