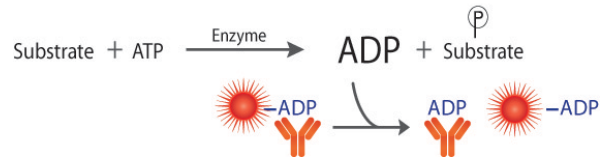
Running Kinase Assays in Kinetic Mode with the Transcreener® ADP² Assay

Meera Kumar
BellBrook Labs, Madison-WI

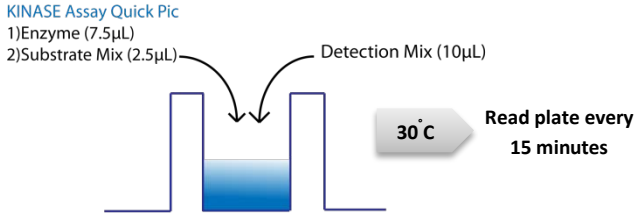
This Application Note describes how to run the Transcreener® ADP² assay in kinetic, or continuous mode. Using this mode of measurement reduces the number of experiments required to optimize conditions for a new enzyme; e.g., incubation time and temperature, enzyme concentration, etc. Running assays in real time also helps save on the cost of reagents and provides a check on assay linearity.

The Transcreener® ADP² Assay relies on direct immunodetection of ADP with a far-red readout in a simple mix-and-read format. It has fewer reagents, fewer assay steps and less chance of interference compared with other methods, all of which rely on coupling and/or reporter enzymes. The assay is available in three different detection modes, fluorescence polarization (FP), fluorescence intensity (FI), and time resolved Forster resonance energy transfer (TR-FRET). All of them use a far red tracer to minimize compound interference. We will use the FP assay as an example in this application note; the methods for the other detection modes are very similar.



The Transcreener ADP2 FP Assay measures the progress of any enzyme that produces ADP. Displacement of the tracer by ADP causes a decrease in fluorescence polarization. The assay uses a red-shifted tracer, which reduces compound interference and provides a robust ratiometric readout.

Shown below is a general protocol for setting up an experiment to run the Transcreener® ADP² assay in kinetic mode. As an example we show here how to run an ABL1 titration to produce reaction progress curves, and data from similar titations with Akt2 and ROCK1 are also shown.



Kinase Reaction Reagents:

1. **Target Protein:** Serially titrate the enzyme Abl1 in 7.5 µL at 2X desired concentration in target buffer containing DMSO. (Note: in a 10 µL enzyme reaction, the Final DMSO concentration will be 1%)
2. **Substrate Mix:** Add 4 µM ATP and 40 µM ABLtide at 4X concentration in the Kinase Buffer. (Note: in a 10 µL enzyme reaction, the final ATP and ABLtide concentration is 1 and 10 µM respectively.
3. **Transcreener ADP² FP Detection Mix:** Add 10 µL of detection mixture containing 4 nM tracer and 1 µg/mL of ADP² antibody. (Note: The Detection Mix is added at time zero, without the Stop and Detect Buffer B)

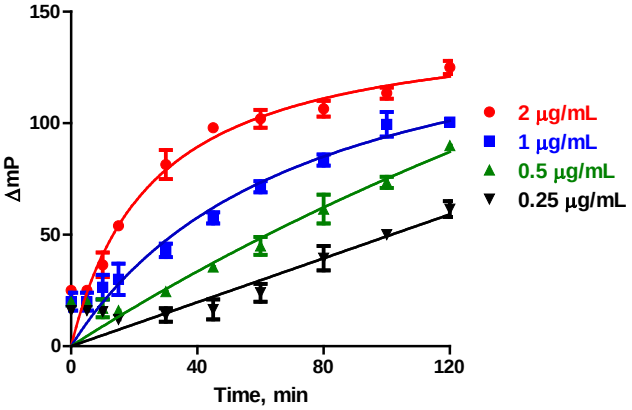
Detection Reagents for other formats:

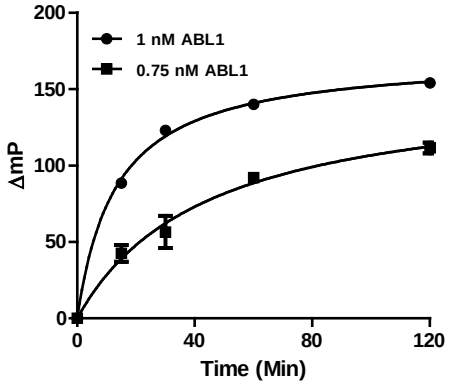
Transcreener ADP² FI Detection Mix: Contains 4 nM tracer, 1 µg/mL ADP² IR dye-QC Antibody.
Transcreener ADP² TR-FRET Detection Mix: Contains 9.7 nM tracer, 4 nM ADP² Tb Antibody.

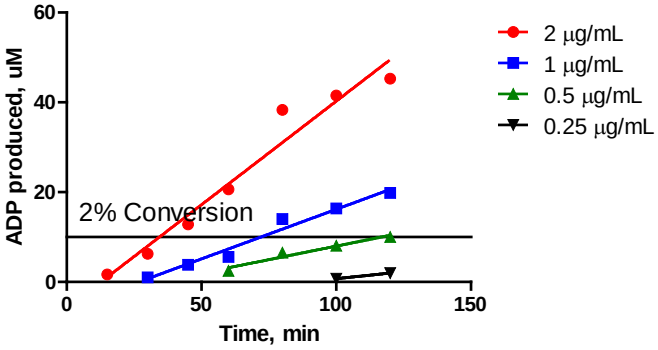
Protocol:

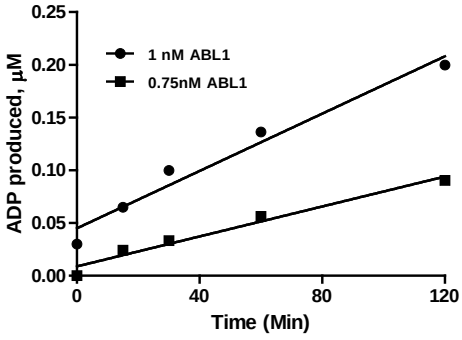
- Serially titrate Abl1 in 7.5 µL to a LV 384 well plate.
- Add 2.5 µL of Substrate Mix (Abltide+ATP) to wells. Addition of substrate initiates the Abl1 reaction. Control reactions without peptide or ATP should also be run to determine any non-specific ATP hydrolysis by Abl1.
- Prepare a 12 point standard curve, mimicking various percent conversions of 1 µM ATP to ADP in buffer containing 10 µM ABLtide. All wells should now have 10 µL reactions.
- Add 10 µL of Detection Mix containing tracer and antibody to all wells. (Do not add Stop and Detect Buffer here)
- Mix plate well and cover the plate before incubating the plate at 30°C.
- Plates are read every 15 minutes for an hour and an hour after that until a good assay window is achieved.

The polarization values obtained from this experiment are fit to a curve using Graph Pad Prism Software. We recommend using the log [Enzyme] versus response with the four parameter fit. One can use a standard curve (see ADP[2] Technical Manual for more information) to extrapolate the raw polarization values to product formed at each time point. The product formed is fit using linear regression in Graph Pad Prism to get a linear correlation between the ADP formed at various ABL1 concentrations.





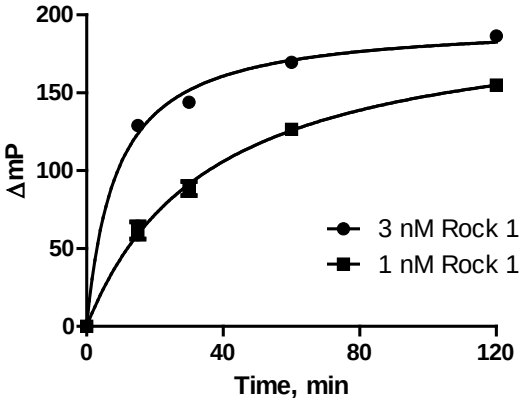


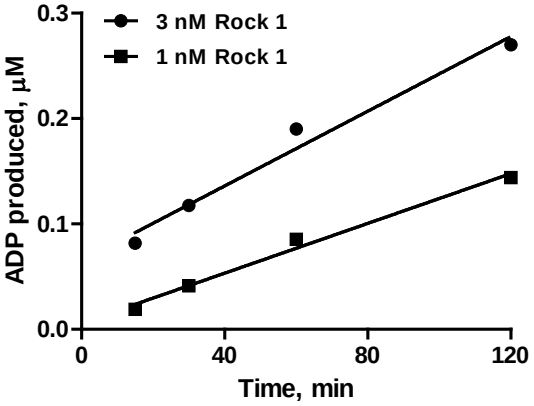


	1 nM ABL1	0.75nM ABL1
R square	0.9659	0.9686

Time course of Abl1 reaction. *1 µM ATP and 10 µM Abltide peptide in a 10 µL enzyme reaction. ΔmP values represent the difference in mP between +/- Abl1 enzymes. (B) Raw polarization values were converted into product formed (ADP, µM) using a standard curve run under similar experimental conditions. The linear correlation between the enzyme concentration and product formation demonstrates initial velocity conditions and adherence to Michaelis-Menten parameters.*

Time course of Akt2 reaction. *500 µM ATP and 150 µM Crosstide peptide in a 10 µL enzyme reaction. ΔmP values represent the difference in mP between +/- Akt2 enzymes. (B) Raw polarization values were converted into product formed (ADP, µM) using a standard curve run under similar experimental conditions. The linear correlation between the enzyme concentration and product formation demonstrates initial velocity conditions and adherence to Michaelis-Menten parameters.*





	3 nM Rock 1	1 nM Rock 1
R square	0.9755	0.9879

Time course of Rock1 reaction*. 1 µM ATP and 10 µM S6 peptide peptide in a 10 µL enzyme reaction. ΔmP values represent the difference in mP between +/- Rock1 enzymes. (B) Raw polarization values were converted into product formed (ADP, µM) using a standard curve run under similar experimental conditions.*

We show a simple protocol here for determining initial progress curves in real time for Kinases using the Transcreener® ADP^2 Assay.

Please visit www.bellbrooklabs.com or contact BellBrook Labs for pricing for the Transcreener® Assays. Custom quotes are available for bulk orders.

Phone Orders: 608.443.2400
Toll-Free: 866.313.7881

Fax Orders: 608.441.2967

Email Orders: info@bellbrooklabs.com

For technical information, please contact:

Meera Kumar, Applications Scientist
Tel: 608.443.2400
Toll-Free: 866.313.7881
Email: meera.kumar@bellbrooklabs.com.

BellBrook Labs