# Generating Better Biology Through the Use of BellBrook Labs' Transcreener® ADP² FP Assay and the BMG LABTECH PHERAstar *Plus*

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## Overview

The Transcreener® ADP² FP Assay is a universal, one-step homogenous assay based on the detection of ADP, which is formed by all kinases and other cellular enzymes. Because the assay detects the invariant nucleotide reaction product of many ATP utilizing enzymes, a single set of detection reagents can be used for drug targets within an enzyme family while using all acceptor substrates. Here we demonstrate the ability to test both the ABL1 tyrosine kinase, and the ABL1 T315I mutant with the Transcreener ADP² assay using BMG LABTECH's PHERAstar *Plus* Microplate Reader in 384-well plate format. The simultaneous dual emission capability of the PHERAstar *Plus*, coupled with its five optimized photomultiplier tubes, shows this reader to be ideal for Transcreener use in HTS labs. Excellent Z´ values are shown at low levels of ATP consumption by converting assay signal to product formation. The expected differences in inhibitor potency were observed using the wild type and mutant ABL kinases.

## Introduction

The drive to find screening assays that are easy to use, cost effective, and biologically relevant is essential in today's market. This is fueled by the ability to use a generic platform for initial rate enzyme reaction kinetics. In addition, there is the need to conserve precious enzyme and acceptor substrates, and to keep the time and cost of assay optimization. BellBrook Labs has developed the Transcreener ADP² FP Assay to meet these needs. The Transcreener ADP² FP Assay is a universal, one-step homogenous assay based on the detection of ADP, which is formed by hundreds of cellular enzymes. Because the assay is able to detect the invariant nucleotide reaction product of ATP utilizing enzymes, a single set of detection reagents can be used for all enzymes in a family and all acceptor substrates.

Here we demonstrate the ability to test both the wild type ABL1 tyrosine kinase and the T315I mutant using the Transcreener ADP² assay on the BMG LABTECH PHERAstar *Plus* Microplate Reader. ABL1 kinase is a therapeutic target for the treatment of Chronic Myeloid Leukemia. As with many cancers, mutations in the target protein confer resistance to treatment, resulting in little to no therapeutic effect. Our work here demonstrates that the Transcreener ADP² Assay can distinguish between these two naturally occurring forms of enzyme. Gleevec®, an inhibitor for the wild type ABL1 kinase, is used to show potency differences between the wild type and the T315I mutant, which is known to show resistance to this compound. A small inhibitor panel is further used to show biological differences in inhibition between the enzymes. The combination of the Transcreener ADP² Assay with the PHERAstar *Plus* provides a solution to researchers looking to screen their compound libraries to find drug candidates that are efficacious against wild type or mutant forms of a drug target.

## The Transcreener® ADP² FP Assay

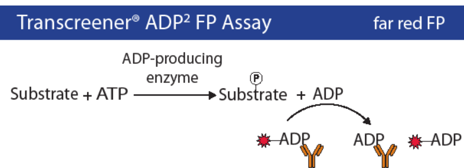


**Figure 1. The Transcreener ADP² Assay.** The Transcreener ADP² Assay was developed to follow the progress of any enzyme that produces ADP. The Transcreener ADP Detection Mixture comprises an ADP Alexa633 Tracer bound to an ADP² antibody. The tracer is displaced by ADP, the invariant product generated during an enzyme reaction. The displaced tracer freely rotates in solution leading to a decrease in fluorescence polarization. Therefore, ADP production is proportional to a decrease in polarization. The far red tracer minimizes interference from fluorescent compounds and light scattering.

## The BMG LABTECH PHERAstar *Plus* HTS Reader

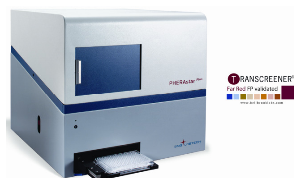


**Figure 2. The PHERAstar *Plus* HTS Microplate Reader.** The PHERAstar *Plus* features full modularity in all detection modes covered by two matched pairs of PMTs and optimized assay specific modules. The PHERAstar *Plus* is enhanced with Advanced Fluorescence Polarization reading capability. Simultaneous dual emission (SDE) detection enables fast read time and the highest sensitivity. The Transcreener FP Application Specific Module is recommended for the ADP² Assay described here. The PHERAstar *Plus* has been validated for use with Transcreener ADP² FP Assay. The conditions for optimal performance in 384-well format with the Transcreener FP module include: 30 flashes and a positioning delay of 0.1 seconds.

## ABL1 and ABL1 T315I Kinase Titrations

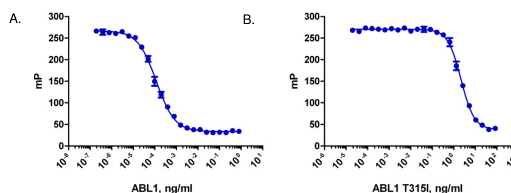


**Figure 3. ABL1 and ABL1 T315I titrations in 384-well format.** The ABL1 and ABL1 T315I serial titrations (1:2) were performed in a 10 µL reaction volume at the ATP $K_m$ (10 µM ATP or 4 µM ATP for wild-type and mutant respectively) with 247 µM Abl cytosolic substrate for one hour at room temperature. An equal volume of ADP Detection Mixture was added to stop the reaction, followed by a one hour equilibration before reading. The final 20 µL assay volume containing the ADP Detection Mixture consisted of 2 nM ADP Alexa633 tracer, 0.5X Stop & Detect Buffer B, 6.4 µg/mL ADP² antibody, and 0.5X enzyme reaction mixture (25mM HEPES pH 7.5, 2 mM $MgCl_2$, 1 mM EGTA, 0.5% DMSO, 0.005% Brij-35, 5 µM or 2 µM ATP, and 124 µM substrate). $EC_{50}$ for ABL1 (A) was 0.1 pg/mL. $EC_{50}$ for ABL1 T315I (B) was 2.2 ng/mL.

## Z´-Factor at Low % ATP Conversion

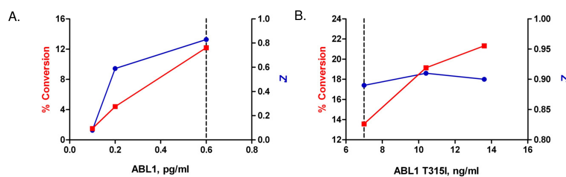


**Figure 4. Excellent Z´ values achieved at low % ATP conversion.** Z´-factor experiments were run to confirm % conversion achieved in the enzyme titration. Enzyme concentrations were chosen as indicated on the graphs above, and reactions were performed under the same conditions used for the enzyme titration. Z´-factor was determined by comparing wells containing enzyme to wells without enzyme. Enzyme concentrations chosen for subsequent inhibitor titration experiments achieved a Z´ ≥ 0.7, highlighted by the dashed lines above. ABL1 (A): 1 pg/mL achieved a Z´= 0.83 at 12% conversion. ABL1 T315I (B): 7 ng/mL achieved Z´= 0.89 at 13% conversion.

## Gleevec® Titration

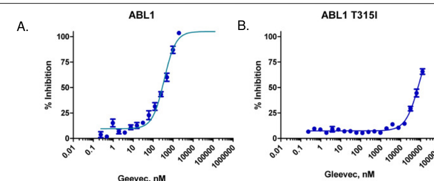


**Figure 5. Increased Gleevec potency of inhibition for ABL1 versus ABL1 T315I.** Gleevec, a known inhibitor of the wild type ABL1 enzyme, and not the mutant ABL1 T315I, was serially titrated into the respective enzyme reaction following the assay conditions noted previously. Results show expected selectivity of Gleevec for ABL1 versus ABL1 T315I, with greater than 1000-fold potency for the wild type enzyme.

## Inhibitor Panel Profile Results

**Figure 6. Potency differences observed between ABL1 and ABL1 T315I using a panel of kinase inhibitors.** In addition to studying Gleevec potency, we also generated an inhibition profile using a panel of tyrosine kinase inhibitors. Inhibitors were serially titrated and added to wild type and mutant enzyme reactions. The color scheme assigned denotes inhibitor potency for each compound. In addition to Gleevec, we show a difference in inhibition potency between ABL1 and ABL1 T315I with other compounds.

## Conclusions

- The BMG LABTECH PHERAstar *Plus* with Transcreener FP Application Specific Filter Module is validated for use with BellBrook Labs' Transcreener ADP² FP Assay for far red detection.
- Wild type and mutant forms of an enzyme, such as ABL1 and ABL1 T315I kinase, can be easily optimized with Transcreener using initial rate conditions (around 10% ATP conversion).
- The Transcreener ADP² FP Assay is an ideal method to screen compound libraries to identify biologically relevant inhibitors for both mutant and wild type versions of a drug target as well as any target within an enzyme family.

For more information on Transcreener validation on BMG LABTECH readers, or for a copy of this poster, please visit BMG at booth # 456.

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