# Volume Scaling of the Transcreener® ADP² FP Assay on the BioTek Synergy™ 2 and 4 Multi-Mode Microplate Readers Allows For Easy Transition From Assay Development to High-Throughput Screening

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## Overview

We present data showing the validation of BellBrook Labs' Transcreener ® ADP² FP Assay on the BioTek Synergy™ 2 and Synergy™ 4 plate readers. The Transcreener ADP² Assay is a universal, second generation far red fluorescence polarization assay that detects the ADP nucleotide by-product from any ATP-utilizing enzyme reaction. The assay is amenable to volume scaling, as shown by data generated in 96-well and 384-well microplate formats. Data generated with a standard curve in both microplate formats resulted in Z´ values ≥ 0.6. Further, the 96-well assay using the standard PMT and the 384-well assay using the red-shifted PMT meet BellBrook Labs' Instrument Validation Program criteria, which include Z´ > 0.7 with Δ mP shift ≥ 95 at 10% conversion of 10 µM ATP to ADP. Further enzyme studies demonstrate that 96- and 384-well plate formats yield excellent Z´-factor results with Protein Kinase A (PKA) at low % ATP conversion. Known PKA inhibitors show comparable potency in both 96- and 384-well plate formats, and between different Synergy plate readers. Although the standard PMT Synergy does not meet validation criteria in 384-well plate format, comparable inhibitor potency results are obtained between the standard and red-shifted PMT instruments.

## Introduction

A critical factor in achieving success with an assay is to ensure that the assay detection instrumentation is compatible with the reagents being used. In addition to overall compatibility with the reader detection mode, assay reagents must also demonstrate scalability from larger volume to smaller volume wells to accommodate higher throughput processing.

We present data showing the validation of BellBrook Labs' Transcreener® ADP² FP Assay on the BioTek Synergy™ 2 and Synergy™ 4 plate readers. The Transcreener ADP² Assay is a universal, second generation far red fluorescence polarization assay that detects the ADP nucleotide by-product from any ATP-utilizing enzyme reaction. The Transcreener Assay has been validated in both 96- and 384-well plate formats on the Synergy 2 and 4 plate readers. Data generated with a standard curve in both microplate formats meet BellBrook Labs' Instrument Validation Program criteria, which include Z´ > 0.7 with Δ mP shift ≥ 95 at 10% conversion of 10 µM ATP to ADP. The 96-well assay met the validation criteria using the standard PMT. Although the 384-well assay performed well and achieved a Z´ ≥ 0.6 using the standard PMT, the validation criteria were met using a red-shifted PMT.

We use protein kinase A (PKA) as an enzyme model to further demonstrate scalability of the Transcreener Assay on BioTek detection instrumentation. Enzyme titrations, Z´-factor determinations, and inhibitor potency studies with PKA in 96- and 384-well formats are shown here. Results indicate that researchers can have confidence in both reagent and reader performance regardless of which well format or detection instrumentation they are using.

## The Transcreener® ADP² FP Assay

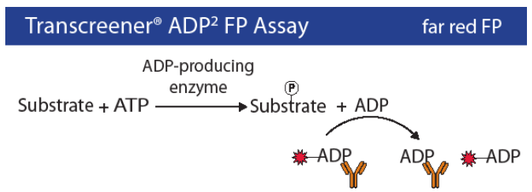


**Figure 1. The Transcreener ADP² Assay.** The Transcreener ADP Detection Mixture comprises an ADP Alexa633 Tracer bound to an ADP² antibody. The tracer is displaced by ADP formed in an enzyme reaction. The displaced tracer freely rotates in solution leading to a decrease in fluorescence polarization. Therefore, ADP production is proportional to a decrease in polarization. The far red tracer in this assay minimizes interference from fluorescent compounds and light scattering.

## The BioTek™ Synergy 2 and 4 Multi-Mode Microplate Readers

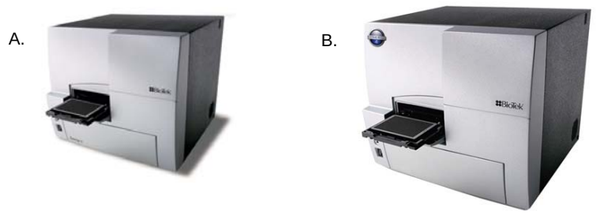


**Figure 2. The Synergy 2 and Synergy 4 Microplate Readers.** The Synergy 2 and 4 plate readers can be used in well formats from 6- to 1536-well plates. Both readers have 2 broad spectrum light sources for fluorescence. Gen5 Data Analysis Software comes standard with both readers. The Synergy 2 (A) can be customized with combinations of monochromator, filters and dichroic mirrors for optimal performance in all detection modes. The Synergy 4 (B) contains Hybrid Technology comprising a high-performance filter system with flexible monochromator detection. For optimal FP performance, an excitation filter at 620/40nm was used, combined with an emission filter at 680/30nm and 660nm cutoff dicroic mirror. The instrument comes with a standard PMT, or optional red-shifted PMT detector which we tested in this study.

## Assay Validation at 10% Conversion

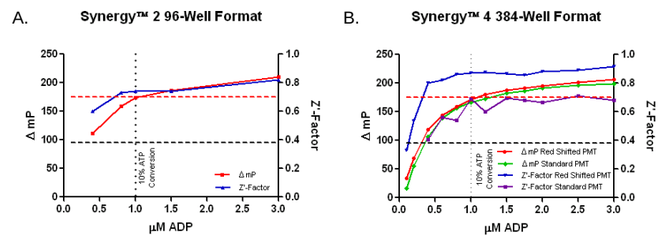


**Figure 3. The Synergy 2 and 4 meet BellBrook Labs' Instrument Validation Criteria.** The validation criteria include: Z´ ≥ 0.7 at 10% conversion (1 µM ADP) of 10 µM ATP, a Δ mP shift ≥ 95 mP at 10% conversion and read time ≤ 5 minutes. The 96-well format assay passed validation under the following conditions: 15 flashes per well, Z´= 0.74, Δ mP of 174 in 1:39 minutes. The 384-well format assay passed validation with the red-shifted PMT under the following conditions: 3 flashes per well, Z´= 0.81, Δ mP of 168 in 1:52 minutes. The 384-well format assay using the standard PMT did not meet the validation criteria of the Program, but results yielded acceptable data by many standards: 12 flashes per well, Z´ ≥ 0.6, Δ mP of 166 in 4:11 minutes.

## Protein Kinase A Enzyme Titrations

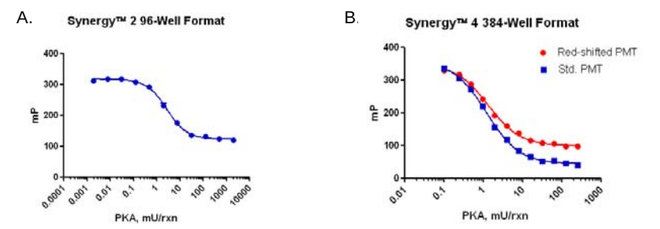


**Figure 4. PKA titrations in 96 and 384-Well plate formats show comparable $EC_{50}$.** PKA was titrated 1:2 and reactions were performed with 10 µM ATP and 50 µM kemptide substrate for one hour. The final 100 µL (96-well) and 20 µL (384-well) assay volume with the Detection Module consisted of 2 nM Alexa633 tracer, 0.5X Stop and Detect Buffer B, 8.5 µg/ml ADP² antibody, and 0.5X enzyme reaction mixture (50 mM HEPES pH 7.5, 2 mM $MgCl_2$, 1 mM EGTA, 0.5% DMSO, 0.01% Brij-35, 20 mM EDTA, 0.5X ATP and substrate). $EC_{50}$ for 96-well assay was 2.8 mU/rxn. $EC_{50}$ for 384-well assay was 1.3 mU/rxn (red-shifted PMT), and 1.2 mU/rxn (standard PMT).

## Z´-Factor at Low % ATP Conversion

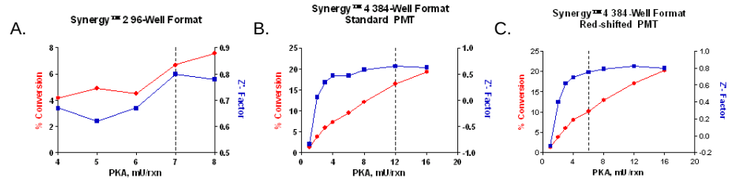


**Figure 5. Excellent Z´ values achieved at low % ATP Conversion.** Z´-factor experiments were run to confirm % conversion achieved in the PKA titration. Enzyme concentrations were chosen as indicated on the graphs above, and reactions were performed under the same conditions used for the enzyme titration. Z´-factor was determined by comparing to a no enzyme control on each plate. Enzyme concentrations chosen for subsequent inhibitor titration experiments achieved a Z´ ≥ 0.7, highlighted by the dashed lines above. 96-well format (A): 7 mU/rxn achieved a Z´= 0.8 at 7% conversion. 384-well format, standard PMT (B): 12 mU/rxn achieved Z´= 0.7 at 16% conversion. 384-well format, red-shifted PMT (C): 6 mU/rxn achieved Z´= 0.8 at 10% conversion.

## Protein Kinase A Inhibitor Potency Study

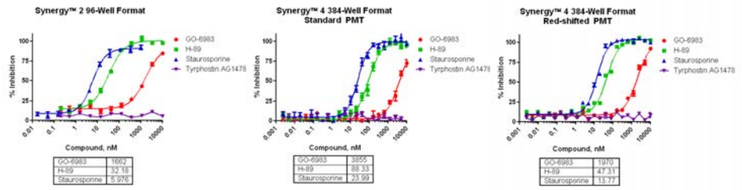


**Figure 6. Potency of PKA inhibitors are comparable between well formats.** GO-6983, H-89, and staurosporine (known PKA inhibitors) and Tyrphostin AG1478 (a no inhibition control for PKA) were serially titrated into the PKA reaction following the assay conditions noted previously for the Z´-factor confirmation study. Results show that the potency of compound inhibition (nM) of the known inhibitors are all comparable between the different well formats tested. There is also good agreement between the inhibitor results obtained in 384-well format using the standard and red-shifted PMT.

## Conclusions

- For optimal performance of Transcreener ADP² Assay on BioTek microplate readers, we recommend the following: 620/40 nm excitation filter, 680/30 nm emission filter, 660 nm cutoff dicroic mirror.
- The Synergy 2 and 4 pass BellBrook Labs' Validation Program in 96-well format under the following conditions: standard PMT, 15 flashes/well to yield Z´> 0.7 in 1:39 minutes.
- The Synergy 2 and 4 pass BellBrook Labs' Validation Program in 384-well format under the following conditions: red-shifted PMT, 3 flashes/well to yield Z´> 0.7 in 1:52 minutes.
- Studies with a PKA model system in 96- and 384-well plate formats show comparable results on the Synergy 2 and 4 microplate readers.

For more information on Transcreener validation on BioTek readers, or for a copy of this poster, please visit BioTek at booth # 443.


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