**Introduction to the Transcreener® ADP2 Assays**

The Transcreener® ADP2 Assay allows the facile detection and screening of established drug targets including protein and lipid kinases, as well as other ATPases by directly measuring ADP formation. BellBrook Labs extends the robust Transcreener technology from red fluorescence polarization (rFP) to include two other red emission spectrum detection modes including time resolved fluorescence energy transfer (TR-FRET) and fluorescence intensity (FI). The result is compatibility with all major instrument platforms providing the flexibility to move from assay development to high throughput screening using a single assay platform. Validated in multiple research workflows, the ADP2 Assays are compatible with most plate readers commonly found both in academic labs and high-throughput screening settings. Each Transcreener ADP2 Assay employs BellBrook Labs’ monoclonal ADP antibody which has ≥100 fold selectivity for ADP vs ATP, providing generic ADP detection at least 0.1 μM to 100 μM ATP at near-velocity conditions (10% ATP conversion). The single step assay directly detects ADP formed in the enzyme reaction which eliminates enzyme coupling requirements and technologies that use coupling enzymes prior to detection of ADP. Here we provide a new EZ protocol which shows a linear relationship between [ATP] and [ADP Antibody].

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**An EZ Format Provides a Simple Way to Started With Any [ATP]**

A) **Transcreener® ADP2® Far Red FP Assay**

B) **Transcreener® ADP2® TR-FRET Red Assay**

C) **Transcreener® ADP2® FL Assay**

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**Figure 1. The Transcreener ADP2 Assays**

The monoclonal antibody-bound tracer is displaced by ADP, the invariant product of all kinase reactions. With each Transcreener Assay, red tracers minimize interference from fluorescent compounds and light scattering. A) The Transcreener ADP2 Far Red FP Assay comprises a AlexaFluor 633 ADP Tracer bound to the ADP Antibody. The displaced tracer freely rotates in solution leading to a decrease in fluorescence polarization, relative to bound tracer. Production of ADP is proportional to a decrease in polarization. B) The Transcreener ADP2 TR-FRET Assay comprises ADP HiLyte647™ Tracer bound to ADP Antibody-Tb. The displaced tracer no longer participates in FRET leading to a decrease in 665 to 620 nm emission ratio, relative to bound tracer. Therefore, ADP production creates a concomitant decrease in the FRET dependent emission ratio. The time-gated nature further minimizes interference from fluorescent compounds and light scatter. C) The Transcreener ADP2 FL Assay comprises a quenched ADP AlexaFluor Tracer bound to the ADP Antibody conjugated to an IRDye® 700 Quantum Dots. The displaced tracer becomes unquenched in solution leading to a positive increase in fluorescence intensity. Therefore, ADP production is proportional to an increase in fluorescence.

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**Figure 2. The EZ protocol can be getting started.**

The Transcreener ADP2 Assays allow detection of ADP in the presence of excess ATP (assuming initial velocity enzyme reaction conditions) using an antibody with a finite selectivity for the dihydroxyimino, the triphosphate.

However, the linear relationship shown here provides an easy method to determine (Detection Reagent) at any [ATP]. A) For FI, the relationship between [ATP] and [ADP Antibody] is linear, the quantity of ADP Antibody for any [ATP] can be determined using equation of line (y = 1.9x + 7.8; where x = [ATP] in the enzyme reaction, y = ADP Antibody (μM) in the ADP Detection Mixture). B) For TR-FRET, the relationship between [ATP] and HiLyte647™ Tracer is linear, the quantity of ADP Tracer for any [ATP] can be determined using equation of line (y = 1.9x + 7.8, where x = [ATP] in the enzyme reaction, y = ADP Antibody (μM) in the ADP Detection Mixture). C) For TR-FRET, the relationship between [ATP] and IRDye® QC-1 for any [ATP] can be determined using equation of line (y = 0.93x + 7.8; where y = [ATP] (μM) in the enzyme reaction, x = [ATP] (μM) in the enzyme reaction, y = IRDye® QC-1 (μM) in the ADP Detection Mixture). The displaced tracer becomes unquenched in solution leading to a positive increase in fluorescence intensity. Therefore, ADP production is proportional to an increase in fluorescence.

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**Figure 3. The robust assay provides a large signal at low percent ATP conversion with excellent Z’**.

Also, the assay provides excellent reagent and signal stability (Z’ shown for both signal and signal stability of up to 24 hours at room temperature using a 10 μM ATP/100 nM ADP standardized curve. A) Standard curves for PKA readout shown for 0.1, 1.0, 10, 100, and 1000 μM ATP. Excellent Z’ values of >0.7 were achieved at 3% ATP conversion for 1 μM, 10 μM, 100 μM, and 1000 μM ATP and 5% conversion for 0.1 μM ATP. B) Standard curves for TR-FRET readout shown for 0.1, 1.0, 10, 100, and 500 μM ATP. Excellent Z’ values of >0.7 were achieved at 3% ATP conversion for 1 μM, 10 μM, 100 μM, and 500 μM ATP; 5% ATP conversion for 1 μM ATP, 10% ATP conversion for 0.1 μM ATP. C) Standard curves for FI readout shown for 0.1, 1.0, 10, and 100 μM ATP; Excellent Z’ values of >0.7 were achieved at 1% ATP conversion for 10 μM, 100 μM ATP and 5% conversion for 0.1 μM and 1 μM ATP.

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**PKA Titration Minimize Enzyme Requirements**

A) 0.1 μM ATP

B) 1.0 μM ATP

C) 10 μM ATP

D) 100 μM ATP

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**Figure 4. The high sensitivity of the Transcreener ADP2 Assay maximizes enzyme costs.**

Graphs show PKA is capable of catalyzing ATP hydrolysis with 400-fold less efficiency, relative to productive phosphorylation. **A** For PKA, [inhibitor] (nM) vs. the triphosphate. **B** For PKA, [inhibitor] (nM) vs. the dihydroxyimino or the triphosphate.

**Figure 5. Accurate pharmacology shown for each Transcreener ADP2 Assay.**

Dose response curves were generated for known PKA inhibitors by serially titrating into the PKA reaction conditions. A) For PKA, EC50 values for Staurosporine, H-89 and Go-6983 were 37 nM, 179 nM and 500 nM respectively. B) For TR-FRET, EC50 values for Staurosporine, H-89 and Go-6983 were 16 nM, 282 mM and 9.4 nM respectively. C) For FL, similar EC50 values are shown using commonly used HTS and academic plate reader instruments (Safire®/SpectraMax® M5) for Staurosporine, H-89 and Go-6983 were 37/32 nM, 165/124 nM and 9.4/7.4 nM respectively.

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**Conclusions**

Transcreener ADP2 Assays: Three detection modes. All major instrument platforms. Generic ADP assay for easy development to HTS. The Transcreener ADP2 Assay is now offered in red emission spectrum readouts for FP, TR-FRET, and FI readouts allowing compatibility with all instrument platforms. Transcreener assays directly detect ADP formed in the enzyme reaction in a single step, which eliminates false positives seen with technologies that use coupling enzymes prior to ADP detection. The near monomolar ADP antibody has increased sensitivity and greater than 100-fold selectivity over ATP which enables its use from 0.1 μM to 1 mM ATP.