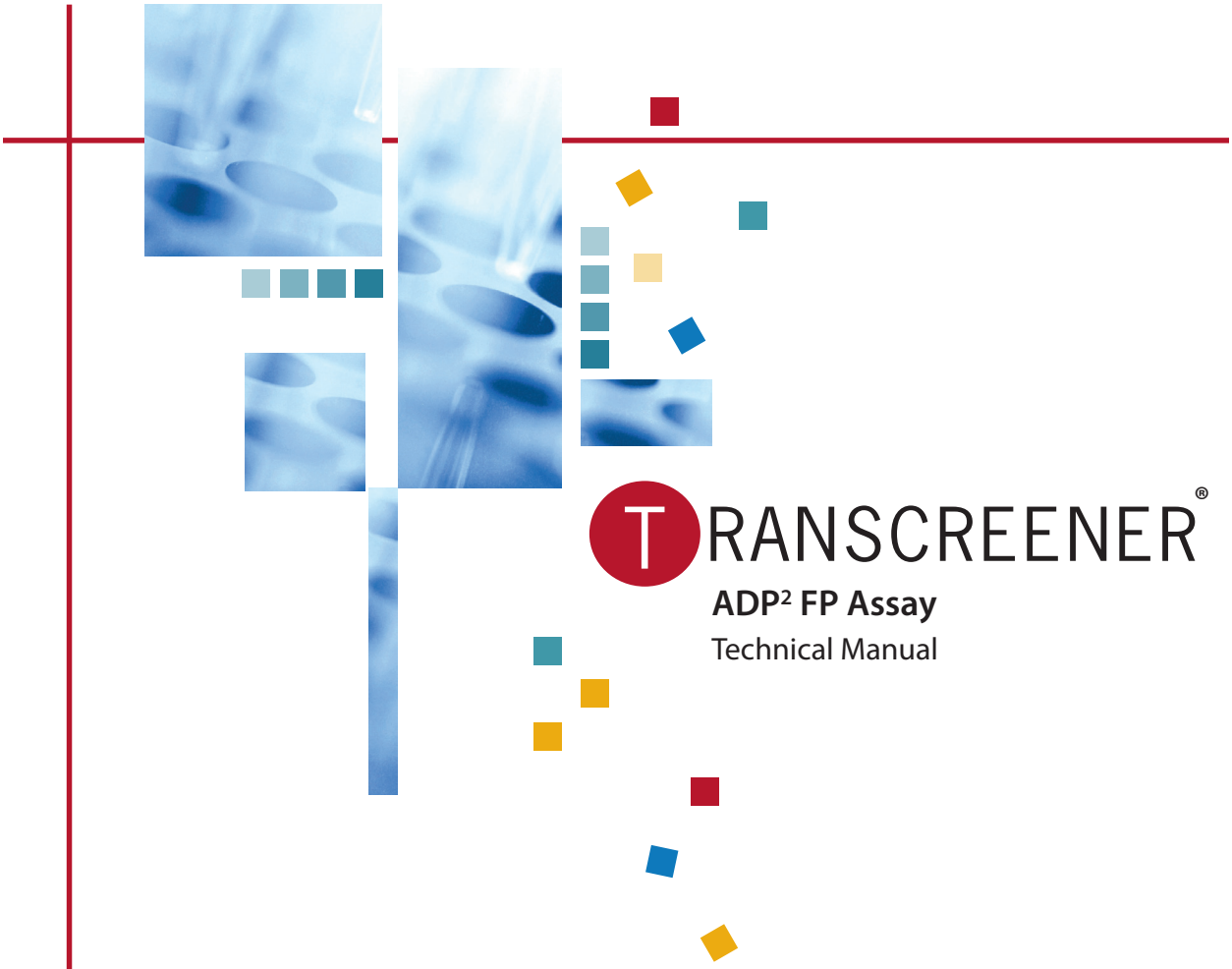
BellBrook Labs

FAR RED FP



TRANSCREENER®

ADP² FP Assay

Technical Manual

Instructions for Part Numbers 3010-1K and 3010-10K

TM#128 • Rev 2023-05

Transcreener® ADP² FP Assay Technical Manual

Contents

1.0 Introduction

The Transcreener® ADP² FP Assay is a far-red, competitive fluorescence polarization (FP) assay (**Figure 1**). Because it is highly selective for ADP, the assay can be used with any enzyme that converts ATP to ADP, regardless of what other substrates are used. Examples of enzymes include protein, lipid, and carbohydrate kinases, ATPases, DNA helicases, carboxylases, and glutamine synthetase.

The Transcreener® assay is designed specifically for high-throughput screening (HTS), with a single-addition, mix-and-read format. It offers reagent stability and compatibility with commonly used multimode plate readers. The generic nature of the Transcreener® HTS assay platform eliminates delays involved in assay development for new HTS targets and greatly simplifies compound and inhibitor profiling across multiple target families.

The Transcreener® ADP² FP Assay provides the following benefits:

- Accommodates ATP concentrations ranging from 0.1 µM to 1,000 µM.
- Excellent data quality (Z' ≥ 0.7) and signal (≥85 mP polarization shift) at low substrate conversion (typically 10% or less) using 1 µM ATP.
- Overcomes the need for time-consuming, one-off assay development for individual members within a group transfer enzyme family by using a single set of assay reagents that detect an invariant product.
- Far-red tracer further minimizes interference from fluorescent compounds and light scattering.

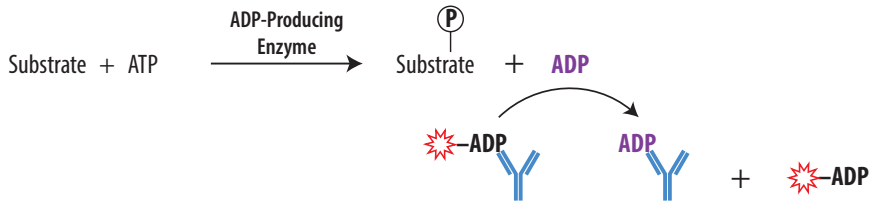


Figure 1. Schematic overview of the Transcreener® ADP² FP Assay. The Transcreener® ADP Detection Mixture contains an ADP Alexa Fluor® 633 tracer bound to an ADP² antibody. ADP produced by the target enzyme displaces the tracer, which rotates freely, causing a decrease in FP.

2.0 Product Specifications

Product	Quantity	Part #
Transcreener® ADP² FP Assay	1,000 assays*	3010-1K
	10,000 assays*	3010-10K

*The exact number of assays depends on enzyme reaction conditions. The kits are designed for use with 384-well plates, using 20 µL reaction volumes.

Storage

Store all reagents at –20°C upon receipt.

2.1 Materials Provided

Component	Composition	Notes
ADP² Antibody	3.1 mg/mL solution in PBS with 10% glycerol*	The concentration of ADP² Antibody needed for an enzyme target is dependent upon the ATP concentration and buffer conditions in the enzyme reaction (see **Section 4.2**). Sufficient antibody is included in the kit to complete 1,000 assays (Part # 3010-1K) or 10,000 assays (Part # 3010-10K) at an ATP concentration up to 100 µM.
ADP Alexa Fluor® 633 Tracer	400 nM solution in 2 mM HEPES (pH 7.5) containing 0.01% Brij-35	The final tracer concentration in the 20 µL reaction is 2 nM.
Stop & Detect Buffer B, 10X	200 mM HEPES (pH 7.5), 400 mM EDTA, and 0.2% Brij-35	The Stop & Detect Buffer B components will stop enzyme reactions that require Mg^{2+}. To ensure that the enzyme reaction is stopped completely, confirm that the EDTA concentration is at least equimolar to the magnesium ion concentration in the reaction. The final concentration of Stop & Detect Buffer B at the time of FP measurement is 0.5X.
ATP	5 mM	The ATP supplied in this kit can be used for the enzyme reaction and to create an ADP/ATP standard curve, if desired.
ADP	5 mM	ADP is used to create the ADP/ATP standard curve.

*The exact concentration may vary from batch to batch. Please refer to the Certificate of Analysis for an accurate concentration.

Caution: ATP is a common reagent in many laboratories; however, it is imperative that a highly purified preparation be used for the Transcreener® assay. If the ATP stock contains impurities, such as ADP, the assay window will be compromised.

2.2 Materials Required but Not Provided

- ***Ultrapure Water***—Some deionized water systems are contaminated with nucleases that can degrade both nucleotide substrates and products, reducing assay performance. Careful handling and use of ultrapure water eliminates this potential problem.
- ***Enzyme***—Transcreener® ADP² assays are designed for use with purified enzyme preparations. Contaminating enzymes, such as phosphatases or nucleotidases, can produce background signal and reduce the assay window.
- ***Enzyme Buffer Components***—User-supplied enzyme buffer components include enzyme, buffer, acceptor substrate, $MgCl_2$ or $MnCl_2$, Brij-35, and test compounds.
- ***Plate Reader***—A multidetection microplate reader configured to measure FP of the Alexa Fluor® 633 tracer is required. The Transcreener ADP² FP Assay has been successfully used on the following instruments: BioTek Synergy™2 and Synergy™4; BMG Labtech PHERAstar Plus and CLARIOstar Plus; Molecular Devices SpectraMax™ Paradigm; Perkin Elmer EnVision and ViewLux; and Tecan Infinite F500, Safire 2™, and M1000.
- ***Assay Plates***—It is important to use assay plates that are entirely black with a nonbinding surface. We recommend Corning® 384-well plates (Cat. # 4514). The suggested plate has a square well top that enables easier robotic pipetting and a round bottom that allows good Z′ factors. It has a recommended working volume of 2–20 µL.
- ***Liquid Handling Devices***—Use liquid handling devices that can accurately dispense a minimum volume of 2.5 µL into 384-well plates.

Note: Contact BellBrook Labs Technical Service for suppliers and catalog numbers for buffer components, and additional information regarding setup of FP instruments.

3.0 Before You Begin

1. Read the entire protocol and note any reagents or equipment needed (see **Section 2.2**).
2. Check the FP instrument and verify that it is compatible with the assay being performed (see **Section 4.1**).

4.0 Protocol

The Transcreener® ADP² FP Assay protocol consists of 4 steps (**Figure 2**). The protocol was developed for a 384-well format, using a 10 µL enzyme reaction and 20 µL complete assay volume at the time that the plates are read. The use of different densities or reaction volumes will require changes in reagent quantities.

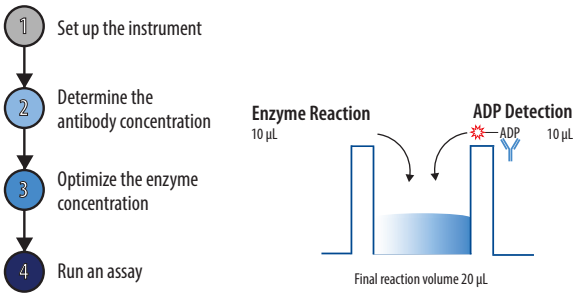


Figure 2. An outline of the procedure. The assay consists of 4 main steps with a mix-and-read format.

4.1 Set Up the Instrument

Becoming familiar with ideal instrument settings for FP is essential to the success of the Transcreener® ADP² FP Assay.

4.1.1 Verify That the Instrument Measures FP

Ensure that the instrument is capable of measuring FP (not simply fluorescence intensity) of Alexa Fluor® 633.

Note: A complete list of instruments and instrument-specific application notes can be found online at: https://www.bellbrooklabs.com/technical-resources/instrument-compatibility Contact BellBrook Labs Technical Service if you have questions about settings and filter sets for a specific instrument.

4.1.2 Define the Maximum mP Window for the Instrument

Measuring high (tracer + antibody) and low (free tracer) FP will define the maximum assay window of your specific instrument. Prepare High and Low FP Mixtures in quantities sufficient to perform at least 6 replicates for each condition.

Use ADP Alexa Fluor® 633 Tracer and Stop & Detect Buffer B at 0.5X concentration in a 20 µL complete assay. This mimics the 2-fold dilution when adding an equal volume of detection mixture to an enzyme reaction. As an example, the 1X ADP Detection Mixture may contain 4 nM tracer. After adding this to the enzyme reaction, the concentration in the 20 µL complete assay would be 2 nM. Use 0.5X ADP² antibody concentration calculated from **Figure 3.** The example here uses 10 µM ATP.

High FP Mixture

Prepare the following High FP Mixture as indicated in the table. Pipette 20 µL of the Total High FP Mixture to each well (from the example: 20 µL from 500 µL). Do not further dilute.

Component	Stock Concentration	Complete Assay Concentration	Example: 25 Assays	Your Numbers
ADP² Antibody	3.1 mg/mL	5.9 µg/mL	0.9 µL	
10X Stop & Detect Buffer B	10X	0.5X	25.0 µL	
ADP Alexa Fluor® 633 Tracer	400 nM	2 nM	2.5 µL	
Water			471.6 µL	
Total			**500.0 µL**	

The assay window will depend upon your initial ATP concentration. These volumes can be adjusted for fewer assays and different ATP concentrations.

Note: The complete assay concentrations with the Stop & Detect Buffers are based on a 20 µL final volume.

Low FP Mixture

Prepare the following Low FP Mixture as indicated in the table. Pipette 20 µL of the Total Low FP Mixture to each well (from the example: 20 µL from 500 µL). Do not further dilute.

Component	Stock Concentration	Complete Assay Concentration	Example: 25 Assays	Your Numbers
10X Stop & Detect Buffer B	10X	0.5X	25.0 µL	
ADP Alexa Fluor® 633 Tracer	400 nM	2 nM	2.5 µL	
Water			472.5 µL	
Total			**500.0 µL**	

Caution: Contact BellBrook Labs Technical Service for assistance if the assay window is <150 mP.

4.1.3 Measure the FP

Subtract the Low FP Mixture readings from the corresponding High FP Mixture readings. The difference between the low and high FP values should be >150 mP.

4.2 Determine the Optimal ADP² Antibody Concentration

The Transcreener® ADP² FP Assay requires detection of ADP in the presence of excess ATP (assuming initial velocity enzyme reaction conditions) using an antibody with a finite selectivity for the diphosphate vs. the triphosphate. The concentration of ADP² Antibody determines the total assay window and the ADP detection range; the amount needed primarily depends upon the ATP concentration in the enzyme reaction. To produce the most sensitive and robust assay signal, it is necessary to perform an ADP² Antibody titration in the buffer system ideal for your enzyme or drug target.

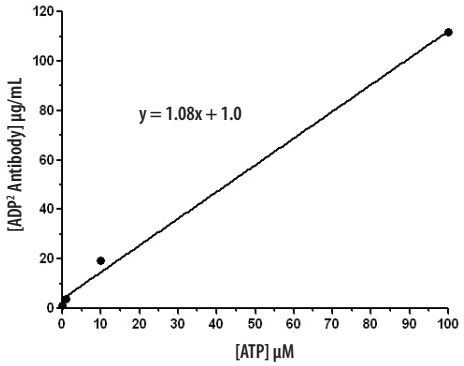


Figure 3. Linear relationship between [ATP] and [ADP² Antibody]. The antibody concentration can be calculated using the equation: $y = 1.08x + 1.0$

4.2.1 Calculate the Antibody Concentration

As shown in **Figure 3**, the relationship between ATP and ADP² Antibody concentrations is linear. (Though shown for 0.1–100 µM ATP, the relationship is valid to 1,000 µM ATP.) Therefore, the quantity of ADP² Antibody for enzyme reactions that use between 0.1 µM and 1,000 µM ATP can be determined using the equation **y = mx + b**, where **x** = [ATP] (µM) in the 10 µL enzyme reaction, **y** = [ADP² Antibody] (µg/mL) in the 1X ADP Detection Mixture, **m** (slope) = 1.08, and **b** (y-intercept) = 1.0. We recommend a 20 µL complete assay for a 384-well plate (10 µL of the 1X ADP Detection mixture added to 10 µL of the enzyme reaction).

For example, if you are using 3 µM ATP in a 10 µL enzyme reaction, the optimal ADP² Antibody concentration in the 10 µL 1X ADP Detection Mixture would be (1.08 × 3) + 1.0 = 4.24 µg/mL. In the 20 µL complete assay, the optimal ADP² Antibody concentration would be half the concentration in the 1X ADP Detection Mixture, or 2.12 µg/mL for this example.

4.2.2 Optimize the Antibody Concentration

Using the ADP² Antibody concentration calculated using the equation in **Figure 3** will produce excellent results for most users. If it does not produce the results you require, refer to **Section 7.1** for instructions on preparing an ADP² Antibody titration in the buffer system ideal for your enzyme target.

4.3 Optimize the Enzyme Concentration

Perform an enzyme titration to identify the optimal enzyme concentration for the Transcreener® ADP² FP Assay. Use enzyme buffer conditions, substrate, and ATP concentrations that are optimal for your target enzyme. If a compound screen is planned, you should include the library solvent at its complete assay concentration. We routinely use enzyme buffer containing 35 mM HEPES (pH 7.5), 4 mM $MgCl_2$, 1% DMSO (test compound solvent), 0.015% Brij-35, and ATP. Run your enzymatic reaction at its requisite temperature and time period. Refer to **Section 7.3** for the tolerance of different components for your buffer conditions.

4.3.1 Enzyme Titration Steps

To achieve the most robust assay and a high signal, the quantity of enzyme required to produce a 50–80% change in FP signal is ideal (EC_{50} to EC_{80}) for screening of large compound libraries and generating inhibitor dose-response curves (see **Figure 4**). To determine the EC_{80} enzyme concentration, use the following equation:

$$EC_X = (X \div (100 - X))^{(1 \div |\text{hillslope}|)} \times EC_{50}$$

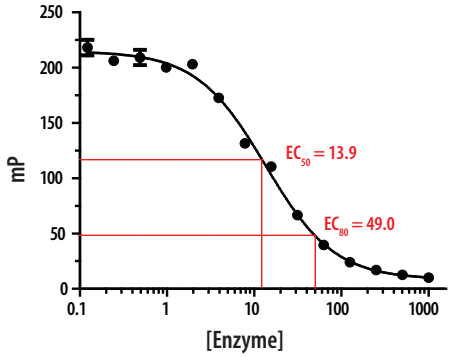


Figure 4. Enzyme titration curve. Titration with the EC_{80} concentration indicated. The EC_{80} may vary based on enzyme lot. Please use C of A for the recommended EC_{80} for your assay.

4.3.2 Enzyme Assay Controls

The enzyme reaction controls define the limits of the enzyme assay.

Component	Notes
0% ATP Conversion Control	This control consists of the ADP Detection Mixture, the enzyme reaction components (without enzyme), and 100% ATP (0% ADP). It defines the upper limit of the assay window.
100% ATP Conversion Control	This control consists of the ADP Detection Mixture, the enzyme reaction components (without enzyme), and 100% ADP (0% ATP). It defines the lower limit of the assay window.
Minus-Nucleotide Control and Minus-Substrate Control	To verify that the enzyme does not interfere with the detection module, perform an enzyme titration in the absence of nucleotide (i.e., ATP) or acceptor substrate.
ADP/ATP Standard Curve	Although optional, an ADP/ATP standard curve can be useful to ensure day-to-day reproducibility and that the assay conditions were performed using initial rates. It can also be used to calculate product formed and inhibitor IC_{50} values. See **Section 7.2** for a description of how to run the standard curve.
Background Control	Use only 0.5X enzyme reaction conditions and Stop & Detect Buffer B.

4.4 Run an Assay

4.4.1 Experimental Samples

1. Add the enzyme reaction mixture to test compounds and mix on a plate shaker.
2. Start the reaction by adding ATP and acceptor substrate, then mix. The final volume of the enzyme reaction mixture should be 10 µL. Incubate at a temperature and time ideal for the enzyme target before adding the ADP Detection Mixture.
3. Prepare the 1X ADP Detection Mixture as follows:

	ATP Concentration: Examples			
Component	**1 µM**	**10 µM**	**100 µM**	**Your Numbers**
ADP² Antibody	6.7 µL	38.1 µL	351.6 µL	
ADP Alexa Fluor® 633 Tracer	100 µL	100 µL	100 µL	
10X Stop & Detect Buffer B	1,000 µL	1,000 µL	1,000 µL	
Water	8,893 µL	8,862 µL	8,548 µL	
Total	**10,000 µL**	**10,000 µL**	**10,000 µL**	

Final concentrations in the 1X ADP Detection mixture should be 4 nM tracer, 1X Stop & Detect Buffer B, and the ADP² Antibody concentration calculated using the equation in **Figure 3**. An example is shown below:

	y = 1.08x + 1.0		
ATP	1 µM	10 µM	100 µM
ADP² Antibody	2.08 µg/mL	11.8 µg/mL	109 µg/mL

4. Add 10 µL of 1X ADP Detection Mixture to 10 µL of the enzyme reaction. Mix using a plate shaker.
5. Incubate at room temperature (20–25°C) for 1 hour and measure FP.

4.4.2 ADP Detection Controls

These controls are used to calibrate the FP plate reader and are added to wells that do not contain enzyme.

Component	Notes
Minus Antibody (Free Tracer) Control	This control contains the ADP Alexa Fluor® 633 Tracer without the ADP² Antibody and is set to 20 mP.
Minus Tracer Control	This control contains the ADP² Antibody without the ADP Alexa Fluor® 633 Tracer and is used as a sample blank for all wells. It contains the same ADP² Antibody concentration in all wells.

5.0 General Considerations

5.1 Assay Types

5.1.1. Endpoint Assay

The Transcreener® ADP² FP Assay is designed for endpoint readout. The Stop & Detect Buffer B contains EDTA to stop Mg^{2+}-dependent enzyme reactions by chelating available Mg^{2+}.

5.1.2 Real-Time Assay

You can perform real-time experiments by substituting the Stop & Detect Buffer B, 10X (provided) with a detection buffer that does not contain EDTA. However, the equilibration time for the tracer and ADP² Antibody is greater than 15 minutes, making it difficult to quantitate ADP produced during short-term enzyme reactions. Note that the optimal ADP² Antibody concentration may change when EDTA is omitted.

5.2 Reagent and Signal Stability

The Transcreener® technology provides a robust and stable assay method to detect ADP.

5.2.1 Signal Stability

The stability of the FP assay window at 10% substrate conversion was determined after the addition of the ADP Detection Mixture to the standard samples. The mP value at 10% substrate conversion (10 µM ATP) remained constant (<10% change) for at least 24 hours at room temperature (20–25°C). If you plan to read FP on the following day, seal the plates to prevent evaporation.

5.2.2 ADP Detection Mixture Stability

The ADP Detection Mixture is stable for at least 8 hours at room temperature (20–25°C) before addition to the enzyme reaction (i.e., when stored on the liquid handling deck).

6.0 Troubleshooting

Problem	Possible Causes and Solutions
Low selectivity	*Suboptimal antibody concentration* • Under the reaction conditions used in the Transcreener® ADP² FP Assay, the ADP² Antibody is >140-fold selective for ADP over ATP. To achieve maximum sensitivity and assay window, the ADP² Antibody concentration must be optimized for each starting ATP concentration. *ATP concentration out of range* • Ensure that the starting ATP concentration is in the range of 1–1,000 µM.
No change in FP observed	*Low antibody/tracer activity* • The tracer and antibody are stable for up to 6 freeze-thaw cycles. For frequent use, aliquot the antibody and tracer and store the aliquots at –20°C. Use a minimum of 20 µL aliquots.
High background signal	*Nonproductive ATP hydrolysis* • Certain kinases catalyze some level of nonproductive ATP hydrolysis, to the extent that water is able to get into the active site. However, the rates are generally low even in the absence of acceptor substrate and are even further reduced when acceptor substrate is present. If you are using the assay to screen for potential acceptor substrates, then background from ATP hydrolysis has to be taken into account on a case-by-case basis. We recommend a "no substrate" control to detect nonproductive ATP hydrolysis. *Interference from impurities* • Since the assay measures ADP production from any source, impurities that cause ADP production—such as a contaminating kinase, phosphatase, or ATPase—will interfere with accurate measurement of the desired kinase activity. Care should be taken to minimize these potential contaminants in both kinase and protein substrate preparations.

7.0 Appendix

7.1 Optimizing the ADP² Antibody Concentration

Using an antibody concentration calculated using the equation in **Figure 4** will produce excellent results for most users. If it does not produce the results you require, we recommend that you perform an ADP² Antibody titration in the buffer system ideal for your enzyme target. This titration will determine the optimal antibody concentration for your assay conditions. The nucleotide substrate concentration in the enzyme reaction generally determines the appropriate concentration of ADP² Antibody. We recommend using the EC_{85} concentration of ADP² antibody to add to the 1X ADP Detection Mixture.

7.1.1 Titrate the ADP² Antibody

1. Prepare the reaction buffer: 35 mM HEPES (pH 7.5), 5 mM $MgCl_2$, and 0.01% Brij-35. Include ATP and substrate but omit the enzyme
2. Dispense 10 µL of the reaction buffer into each well of columns 2–24.
3. Dispense 20 µL of ADP² Antibody (at 2 mg/mL concentration in the same reaction buffer) into each well of column 1.
4. Remove 10 µL from each well of column 1 and add it to the corresponding well of column 2.
5. Repeat step 4 for the remaining columns, thereby performing a 2-fold serial dilution across the plate to column 24.
6. Add 10 µL of ADP Alexa Fluor® 633 Tracer (to a final concentration of 4 nM) in 1X Stop & Detect Buffer B to each well.
7. Mix the plate, equilibrate at room temperature for 1 hour, and measure FP.

7.1.2 Calculate the Optimal ADP² Antibody Concentration

The antibody concentration at the EC_{85} is often used as a good compromise between sensitivity and maximal polarization value. The EC_{85} is determined by inputting the EC_{50} and hillslope values from a sigmoidal dose-response curve fit into the equation below. The ADP² Antibody is added to the 1X ADP Detection Mixture at a concentration equivalent to 2 x [EC_{85}].

$$EC_{85} = (85 \div (100 - 85))^{(1 \div |\text{hillslope}|)} \times EC_{50}$$

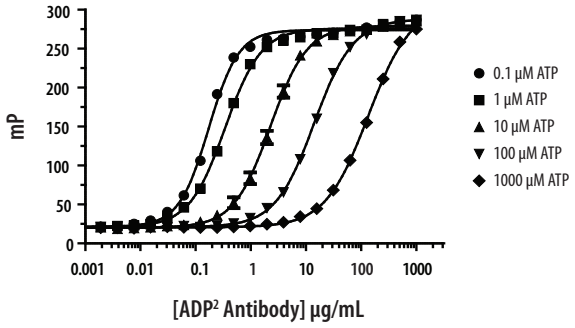


Figure 5. ADP² Antibody titration at various ATP concentrations. The 20 µL complete assay consisted of 2 nM ADP Alexa Fluor® 633 Tracer, 0.5X Stop & Detect Buffer B, 0.5X enzyme reaction mixture (35 mM HEPES [pH 7.5], 2 mM $MgCl_2$, 0.5% DMSO, 0.015% Brij-35, and ATP), and ADP² Antibody (n=3).

7.2 ADP/ATP Standard Curve

The standard curve mimics an enzyme reaction (as ATP concentration decreases, ADP concentration increases); the adenine nucleotide concentration remains constant. The ADP/ATP standard curve allows calculation of the concentration of ADP produced in the enzyme reaction and, therefore, the % ATP consumed (% ATP conversion). In this example, a 12-point standard curve was prepared using the concentrations of ADP and ATP shown in **Table 1**. Commonly, 8- to 12-point standard curves are used.

% Conv.	ATP (µM)	ADP (µM)
100	0	100
50	50	50
25	75	25
15	85	15
10	90	10
7.5	92.5	7.5
5.0	95.0	5.0
3.0	97.0	3.0
2.0	98.0	2.0
1.0	99.0	1.0
0.5	99.5	0.5
0	100	0

Table 1. Concentrations of ATP/ADP to prepare a 12-point standard curve.

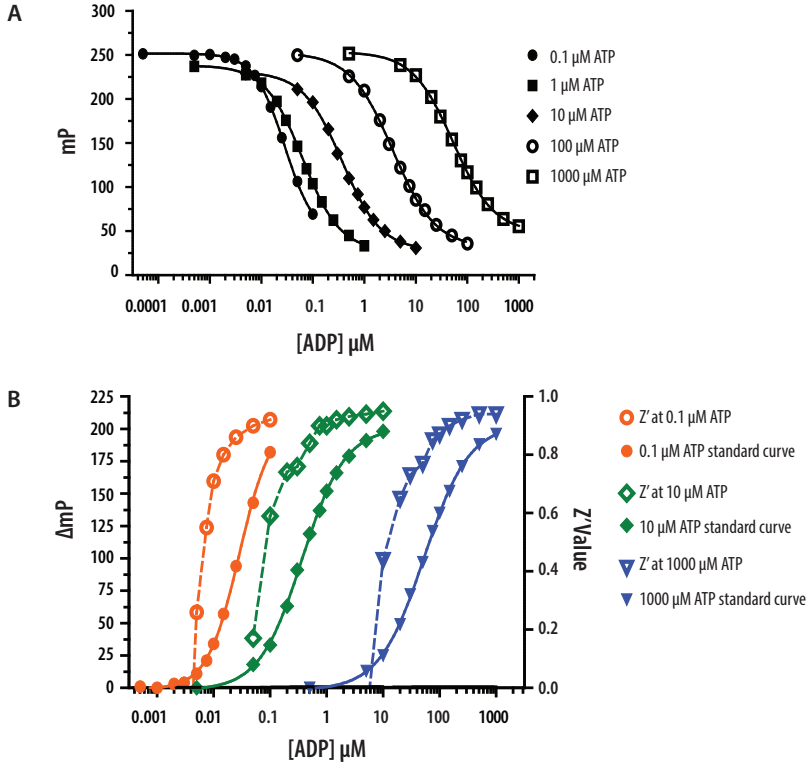


Figure 6. ATP/ADP standard curves. A) Sample data for 0.1 µM, 1 µM, 10 µM, 100 µM, and 1,000 µM ADP/ATP standard curves. The nucleotide concentration reflects the amount in the enzyme reaction, prior to the addition of the 1X ADP Detection Mixture. Curves are obtained in a final 20 µL assay volume consisting of 35 mM HEPES (pH 7.5), 2 mM $MgCl_2$, 1 mM EGTA, 0.5% DMSO, 0.015% Brij-35, 20 mM EDTA, 2 nM Alexa Fluor® 633 Tracer, ADP/ATP standards, and ADP² Antibody (EC_{85} concentration) (n = 24). The data are plotted as mP vs. log [ADP] using 4-parameter nonlinear regression curve fitting. Alternatively, a 2-phase exponential decay and nonlinear regression can be used to present the data (GraphPad Prism). **B)** Excellent Z' values are obtained at <10% ATP conversion for the range of ATP concentrations used. Shown are 0.1 µM, 10 µM, and 1,000 µM ATP standard curves.

Use the following equations to calculate the Z' factor:

$$\Delta mP = mP_{\text{initial [ATP]}} - mP_{\text{sample}}$$

$$Z' = 1 - \frac{[(3 \times SD_{\text{initial [ATP]}}) + (3 \times SD_{\text{sample}})]}{|(mP_{\text{initial [ATP]}}) - (mP_{\text{sample}})|}$$

7.3 Summary of Additive Effects on the Transcreener® ADP² FP Assay

The assay window at 10% substrate conversion (10 µM ATP) remains constant (<10% change) when up to 10% DMSO, DMF, ethanol, acetonitrile, ethanol, or methanol are used in the enzyme reaction. Contact BellBrook Labs Technical Service for further reagent compatibility information.

Component	5-Hour Tolerance (0–100% Conversion Signal)[a]	5-Hour Tolerance (0–10% Conversion Signal)[b]
Solvents		
Acetonitrile	>50%	>50%
DMSO	25.00%	1.56%
DMF	12.50%	6.25%
Ethanol	>50%	25.00%
Methanol	>50%	>50%
Glycerol	>50%	6.30%
Detergents		
Brij-35	0.47%	0.06%
CHAPS	0.16%	0.04%
NP40	0.08%	0.08%
SDS	0.08%	0.02%
Triton X-100	0.08%	0.08%
Sodium deoxycholate	0.16%	0.01%
N-lauroyl sarcosine	0.08%	0.01%
Metal chelates		
EDTA	>250 mM	15.6 mM
EGTA	>250 mM	16.6 mM
Reductants		
Beta mercaptoethanol	3.13%	1.60%
Dithiothreitol	250 mM	125 mM
Salts		
Ammonium acetate	>500 mM	16.3 mM
Ammonium sulfate	31.3 mM	7.8 mM
Calcium chloride	15.6 mM	7.8 mM
Magnesium acetate	125 mM	7.8 mM
Magnesium chloride	62.5 mM	31.3 mM
Magnesium sulfate	15.6 mM	3.9 mM
Manganese chloride	15.6 mM	15.6 mM
Potassium chloride	>1000 mM	62.5 mM
Sodium azide	5.00%	0.63%
Sodium bromide	>1000 mM	125 mM
Sodium chloride	1250 mM	78.1 mM

Component	5-Hour Tolerance (0–100% Conversion Signal)[a]	5-Hour Tolerance (0–10% Conversion Signal)[b]
Phosphatase Inhibitors		
Glycerol phosphate	62.5 mM	15.6 mM
Imidazole	>1000 mM	125 mM
Sodium fluoride	31.3 mM	15.6 mM
Sodium molybdate	250 mM	125 mM
Sodium tartrate	>400 mM	50 mM
Sodium orthovanadate	31.3 mM	0.39 mM
Sodium pyrophosphate	1.6 mM	7.8 mM
Carrier Proteins/Coactivators		
BSA	0.625 mg/mL	0.16 mg/mL
BGG	0.01 mg/mL	0.001 mg/mL
Calmodulin	0.16 mg/mL	0.08 mg/mL

a. <10% drop in ΔmP observed at the listed concentration and below.

b. mP at 0% or 10% increased or decreased <3 standard deviations of the plate controls at the listed concentration and below.

Not all combination of these components have been tested together. Results may vary depending on your assay conditions.

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