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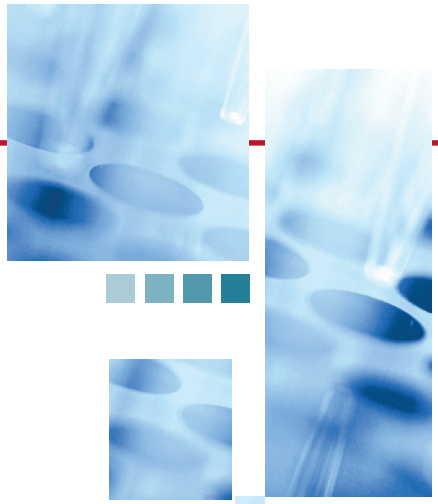

TRANSCREENER®

GDP FP Assay

Technical Manual

Instructions for Part Numbers 3009-1K and 3009-10K

TM#129 • Rev 2025-02

Transcreener® GDP FP Assay Technical Manual

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U.S. Patent 7,332,278, 7,355,010 and 7,378,505 issued. U.S. Patent Application Nos. 11/353,500, 11/958,515 and 11/958,965, U.S. Divisional Application 12/029,932, and International Patent Application Nos. PCT/US07/088111, European Application Nos. 04706975.2 and 05785285.7, Canadian Application 2,514,877, and Japanese Application 2006-503179 applied. The purchase of this product conveys to the buyer the non-transferable right to use the purchased amount of the product and components of the product in research conducted by the buyer (whether the buyer is an academic or for-profit entity). The buyer cannot sell or otherwise transfer (a) this product (b) its components or (c) materials made using this product or its components to a third party or otherwise use this product or its components or materials made using this product or its components for Commercial Purposes other than use of the product or its components to provide a service, information, or data. Commercial Purposes means any activity by a party for consideration other than use of the product or its components to provide a service, information, or data and may include, but is not limited to: (1) use of the product or its components in manufacturing; (2) use of the product or its components for therapeutic, diagnostic or prophylactic purposes; or (3) resale of the product or its components, whether or not such product or its components are resold for use in research. BellBrook Labs LLC will not assert a claim against the buyer of infringement of the above patents based upon the manufacture, use, or sale of a therapeutic, clinical diagnostic, vaccine or prophylactic product developed in research by the buyer in which this product or its components was employed, provided that neither this product nor any of its components was used in the manufacture of such product. If the purchaser is not willing to accept the limitations of this limited use statement, BellBrook Labs LLC is willing to accept return of the product with a full refund. For information on purchasing a license to this product for purposes other than research, contact Licensing Department, BellBrook Labs LLC, 1232 Fourier Drive, Suite 115, Madison, Wisconsin 53717. Phone (608)443-2400. Fax (608)441-2967.

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1.0 Introduction

The Transcreener® GDP FP Assay is a far-red, competitive fluorescence polarization (FP) assay (**Figure 1**). Because it is highly selective for GDP, the assay can be used with any enzyme that converts GTP to GDP, regardless of what other substrates are used. Examples of enzymes include GTPases, such as CDC42, and fucosyltransferases.

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® GDP FP Assay provides the following benefits:

- Accommodates GTP concentrations ranging from 1 µM to 1,000 µM.
- Excellent data quality (Z′ ≥ 0.7) at low substrate conversion (typically 10–30%).
- 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.

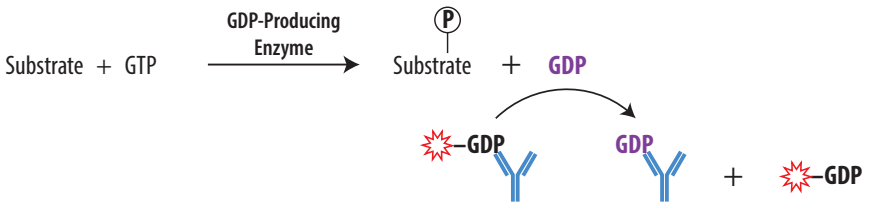

Figure 1. Schematic overview of the Transcreener® GDP FP Assay. The Transcreener® GDP Detection Mixture contains a GDP Alexa Fluor® 633 tracer bound to a GDP antibody. GDP produced by the target enzyme displaces the tracer, which rotates freely, causing a decrease in FP.

2.0 Product Specifications

Product	Quantity	Part #
Transcreener® GDP FP Assay	1,000 assays*	3009-1K
	10,000 assays*	3009-10K

*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.

IMPORTANT: Antibody centrifugation is required to remove aggregates that can disrupt data quality. Antibodies should be centrifuged at 10,000 x g for 10 minutes before use. Following centrifugation, pipet the solution needed from the top of the aliquot to ensure precipitate is not present in the detection reagents.

Storage

Store all reagents at –20°C upon receipt.

2.1 Materials Provided

Component	Composition	Notes
GDP Antibody	3.1 mg/mL solution in PBS with 10% glycerol*	The concentration of GDP Antibody needed for an enzyme target is dependent upon the GTP 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 # 3009-1K) or 10,000 assays (Part 3009-10K) at a GTP concentration up to 100 µM.
GDP 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.
GTP	5 mM	The GTP supplied in this kit can be used for the enzyme reaction and to create a GDP/GTP standard curve, if desired.
GDP	5 mM	GDP is used to create the GDP/GTP standard curve.

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

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

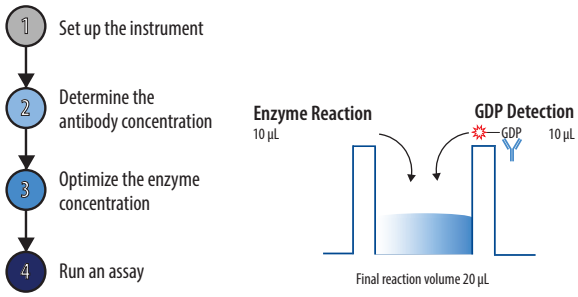

Figure 2. An outline of the procedure. The assay consists of 4 main steps with a mix-and-read format.

4.1 Set Up the Instrument

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

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
GDP Antibody	3.1 mg/mL	20 µg/mL	3.2 µL	
10X Stop & Detect Buffer B	10X	0.5X	25.0 µL	
GDP Alexa Fluor® 633 Tracer	400 nM	2 nM	2.5 µL	
Water			469.3 µL	
Total			**500.0 µL**	

The assay window will depend upon your initial GTP concentration. These volumes can be adjusted for fewer assays and different GTP 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	
GDP 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 GDP Antibody Concentration

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

4.2.1 Titrate the GDP Antibody

1. Prepare the reaction buffer: 35 mM HEPES (pH 7.5), 5 mM $MgCl_2$, and 0.01% Brij-35. Include GTP 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 GDP Antibody (at 3.1 mg/mL 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 GDP 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.

4.2.2 Calculate the Optimal GDP 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 GDP Antibody is added to the 1X GDP Detection Mixture at a concentration equivalent to 2 x [EC_{85}].

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

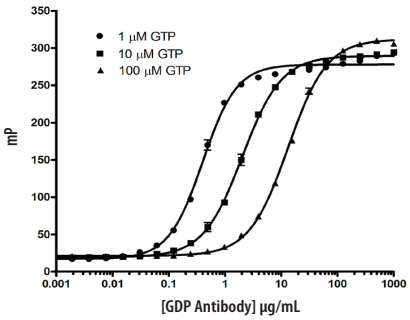

Figure 3. GDP Antibody titration at various GTP concentrations. The 20 µL complete assay consisted of 2 nM GDP Alexa Fluor® 633 Tracer, 0.5X Stop & Detect Buffer B, 0.5X enzyme reaction mixture (25 mM HEPES [pH 7.5], 2 mM $MgCl_2$, 1 mM EGTA, 0.5% DMSO, 0.01% Brij-35, and GTP), and GDP Antibody (n = 3).

4.3 Optimize the Enzyme Concentration

Choose enzyme buffer conditions and GDP Detection Mixture that are ideal for your enzyme target. Perform the enzymatic reaction at its requisite temperature and optimal duration. You can perform an enzyme titration to identify the optimal enzyme concentration for the Transcreener® GDP FP Assay. We routinely use enzyme buffer containing 50 mM HEPES (pH 7.5), 4 mM $MgCl_2$, 1% DMSO (test compound solvent), 0.01% Brij-35, and varying GTP concentrations (1–1,000 µM). 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:

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

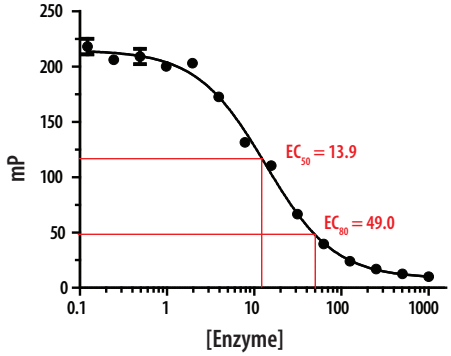

Figure 4. Enzyme titration curve. Titration with the EC_{80} concentration indicated. The EC_{80} may vary based on enzyme lot. Please use C of A for the recommended EC_{80} for your assay.

4.3.2 Enzyme Assay Controls

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

Component	Notes
0% GTP Conversion Control	This control consists of the GDP Detection Mixture, the enzyme reaction components (without enzyme), and 100% GTP (0% GDP). It defines the upper limit of the assay window.
100% GTP Conversion Control	This control consists of the GDP Detection Mixture, the enzyme reaction components (without enzyme), and 100% GDP (0% GTP). 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., GTP) or acceptor substrate.
GDP/GTP Standard Curve	Although optional, a GDP/GTP 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 7.1** 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 GTP, 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 1X GDP Detection Mixture.
3. Prepare 1X GDP Detection Mixture as follows:

	Stock Concentration	Detection Mixture Concentration	Example	Your Numbers
GDP Antibody	3.1 mg/mL	5 µg/µL	16.1 µL	
GDP Alexa Fluor® 633 Tracer	400 nM	4 nM	100 µL	
10X Stop & Detect Buffer B	10X	1X	1,000 µL	
Water			8,883.9 µL	
Total			**10,000 µL**	

Final concentrations in the 1X GDP Detection Mixture should be 4 nM tracer, 1X Stop & Detect Buffer B, and 2 × [EC_{85}] GDP Antibody concentration as determined in **Section 4.2**.

4. Add 10 µL of 1X GDP 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 GDP 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 GDP Alexa Fluor® 633 Tracer without the GDP Antibody and is set to 20 mP.
Minus Tracer Control	This control contains the GDP Antibody without the GDP Alexa Fluor® 633 Tracer and is used as a sample blank for all wells. It contains the same GDP Antibody concentration in all wells.

5.0 General Considerations

5.1 Assay Types

5.1.1. Endpoint Assay

The Transcreener® GDP 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+}. The activity of some GTPase enzymes that produce GDP but do not require metal ions will not be stopped by the addition of the buffer provided.

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 GDP Antibody is approximately 1 hour, making it difficult to quantitate GDP produced during short-term enzyme reactions. Note that the optimal GDP 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 GDP.

5.2.1 Signal Stability

The stability of the FP assay window at 10% substrate conversion was determined after the addition of the GDP Detection Mixture to the standard samples. The mP value at 10% substrate conversion (10 µM GTP) 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 GDP Detection Mixture Stability

The GDP Detection Mixture is stable for at least 24 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® GDP FP Assay, the GDP antibody is >140-fold selective for GDP over GTP. To achieve maximum sensitivity and assay window, the GDP Antibody concentration must be optimized for each starting GTP concentration. *GTP concentration out of range* • Ensure that the starting GTP 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 10 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 GTP hydrolysis* • Certain enzymes catalyze some level of nonproductive GTP 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 GTP hydrolysis has to be taken into account on a case-by-case basis. We recommend a "no substrate" control to detect nonproductive GTP hydrolysis. *Interference from impurities* • Since the assay measures GDP production from any source, impurities that cause GDP production—such as a contaminating kinase, phosphatase, or GTPase—will interfere with accurate measurement of the desired GTPase activity. Care should be taken to minimize these potential contaminants in both GTPase and protein substrate preparations.

7.0 Appendix

7.1 GDP/GTP Standard Curve

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

Table 1. Concentrations of GTP/GDP to prepare a 12-point standard curve.

% Conv.	GTP (µM)	GDP (µM)
100	0	100
60	40	60
40	60	40
30	70	30
20	80	20
15	85	15
10	90	10
8	92	8
6	94	6
4	96	4
2	98	2
0	100	0

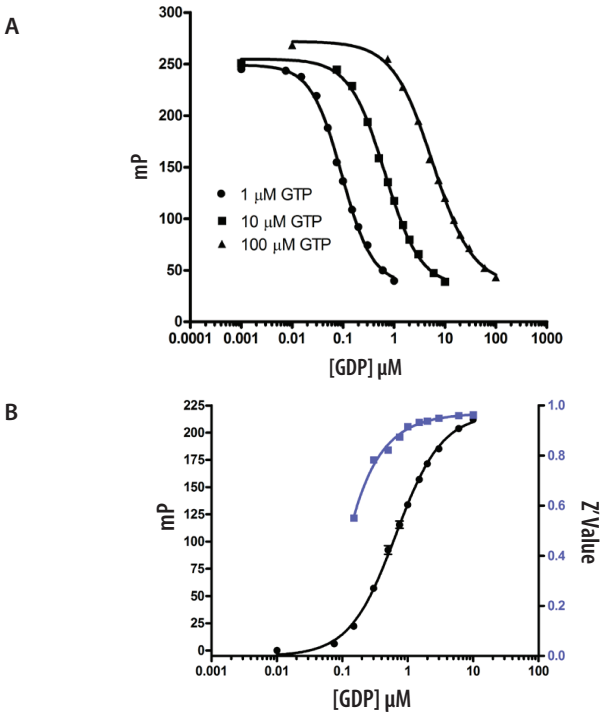

Figure 5. GTP/GDP standard curves. **A)** Sample data for 1 µM, 10 µM, and 100 µM GDP/GTP standard curves. The nucleotide concentration reflects the amount in the enzyme reaction, prior to the addition of the 1X GDP Detection Mixture. Curves are obtained in a 20 µL complete assay consisting of 50 mM HEPES (pH 7.5), 2 mM $MgCl_2$, 1 mM EGTA, 0.5% DMSO, 0.01% Brij-35, 20 mM EDTA, 2 nM GDP Alexa Fluor® 633 Tracer, GDP/GTP standards, and GDP Antibody (EC_{85} concentration) (n = 24). The data are plotted as mP vs. log [GDP] 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% GTP conversion for the 10 µM GTP standard curve.

Use the following equations to calculate the Z′ factor:

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

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

7.2 Summary of Additive Effects on the Transcreener® GDP 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

Component	5-Hour Tolerance (0–100% Conversion Signal)[a]	5-Hour Tolerance (0–10% Conversion Signal)[b]
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
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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