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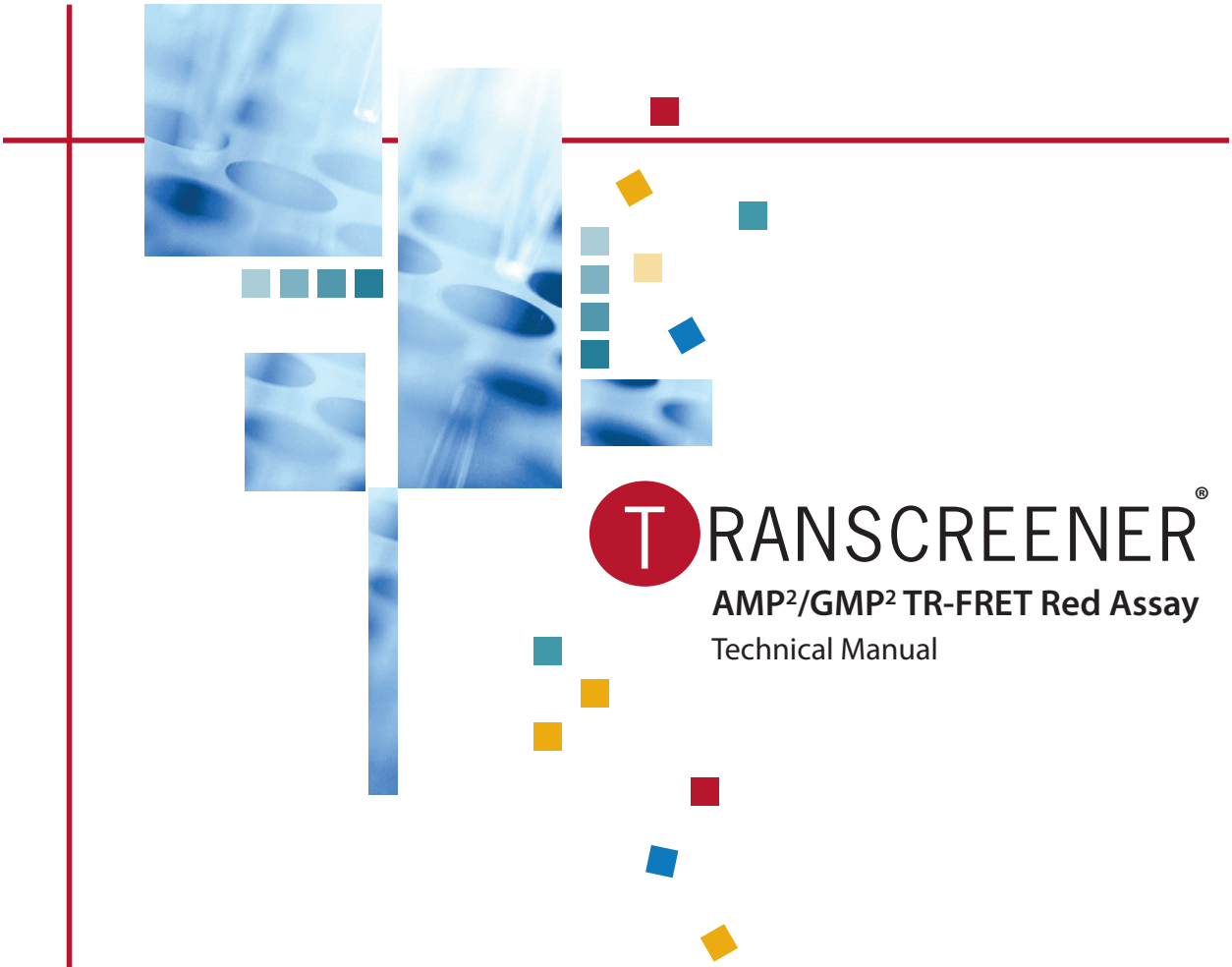
BellBrook Labs

FAR RED TR-FRET



# TRANSCREENER®

## AMP²/GMP² TR-FRET Red Assay

Technical Manual

Instructions for Part Numbers 3020-1K and 3020-10K

TM#127 • Rev 2017-07

# Transcreener® AMP²/GMP² TR-FRET Red Assay Technical Manual

## Contents

## 1.0 Introduction

The Transcreener® AMP²/GMP² TR-FRET Red Assay is a competitive immunoassay for enzymes that produce AMP or GMP with a far-red, time-resolved Förster-resonance-energy-transfer (TR-FRET) readout. The assay relies on a highly specific monoclonal antibody that recognizes AMP or GMP with more than 1,000-fold selectivity over substrate nucleotides, including ATP, cAMP, or cGMP. Enzymes that can be used with the assay include ubiquitin, SUMO, nucleic acid and protein ligases, phosphodiesterases (PDEs), and synthetases.

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® AMP²/GMP² TR-FRET Red Assay provides the following benefits:

- Accommodates substrate concentrations ranging from 0.1 µM to 1,000 µM.
- Excellent data quality (Z′ ≥ 0.7) at low substrate conversion (typically 10–30%).
- The only assay method that allows detection of unmodified AMP and GMP without using coupling enzymes.
- 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.
- Time-gated detection method largely eliminates interference that can result from prompt fluorescence of test compounds.
- Far-red tracer further minimizes interference from fluorescent compounds and light scattering.

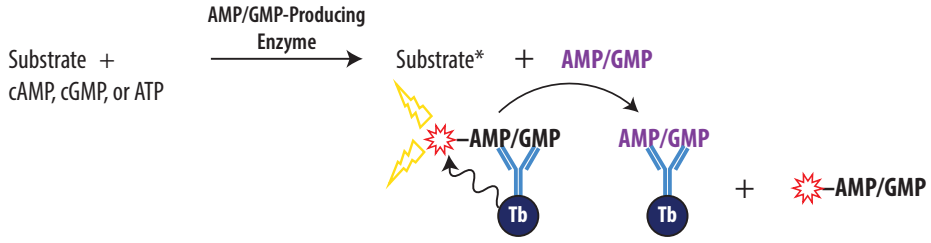



**Figure 1. Schematic overview of the Transcreener® AMP²/GMP² TR-FRET Red Assay.** The Transcreener® AMP/GMP Detection Mixture contains an AMP/GMP HiLyte647 tracer bound to an AMP²/GMP² antibody conjugated to terbium (Tb). Excitation of the Tb complex in the UV range (~330 nm) results in energy transfer to the tracer and emission at a higher wavelength (665nm) after a time delay. AMP/GMP produced by the target enzyme displaces the tracer, which causes a decrease in TR-FRET.

## 2.0 Product Specifications

| Product | Quantity | Part # |
|---|---|---|
| Transcreener® AMP²/GMP² TR-FRET Red Assay | 1,000 assays* | 3020-1K |
| | 10,000 assays* | 3020-10K |

*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 |
|---|---|---|
| AMP/GMP HiLyte647 Tracer | 10 µM solution in 2 mM HEPES (pH 7.5) containing 0.01% Brij-35 | The concentration of AMP/GMP HiLyte647 Tracer needed for an enzyme target depends upon the initial ATP/cAMP/cGMP concentration and buffer conditions in the enzyme reaction (see Section 4.2). Sufficient tracer is included in the kit to complete 1,000 assays (Part # 3021-1K) or 10,000 assays (Part # 3021-10K) at an ATP/cAMP/GMP concentration up to 100 µM. |
| AMP²/GMP² Antibody-Terbium Conjugate | 800 nM solution in HEPES-buffered saline | The final antibody concentration in the reaction is 4 nM in a 20 µL final reaction volume. |
| Tris Solution | 1 M Tris (pH 7.5) | Tris Solution is used to buffer the Detection Mixture. |
| AMP | 5 mM | AMP is used to create an ATP/AMP or cAMP/AMP standard curve. |
| GMP | 5 mM | GMP is used to create a cGMP/GMP standard curve. |

**Caution:** AMP and GMP are common reagents in many laboratories; however, it is imperative that highly purified preparations be used for the Transcreener® assay. If the AMP or GMP stocks contain impurities, 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® AMP/GMP 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, enzyme cofactors, substrates, and test compounds.
- ***Plate Reader***—A microplate reader configured to measure TR-FRET of the Tb:HiLyte647 donor:acceptor pair is required. This assay has been designed to provide high-quality data on any HTS-qualified instrument configured to measure TR-FRET using standard europium or terbium complexes with emission wavelengths at 615 nm and 665 nm. Validation was completed using PHERAstar Plus $Ex_{337}/Em_{620}/Em_{665}$ (BMG LABTECH) and Envision $Ex_{320}/ Em_{615}/Em_{665}$ (Perkin Elmer).
- ***Assay Plates***—It is important to use assay plates that are entirely white with a nonbinding surface. We recommend Corning® 384-well plates (Cat. # 4513).
- ***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 TR-FRET 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 TR-FRET instrument and verify that it is compatible with the assay being performed (see **Section 4.1**).

## 4.0 Protocol

The Transcreener® AMP²/GMP² TR-FRET Red Assay protocol consists of 4 steps (**Figure 2**). The protocol was developed for a 384-well format, using a 15 µL enzyme reaction and 20 µL final volume at the time that the plates are read. The use of different densities or reaction volumes will require changes in reagent quantities.

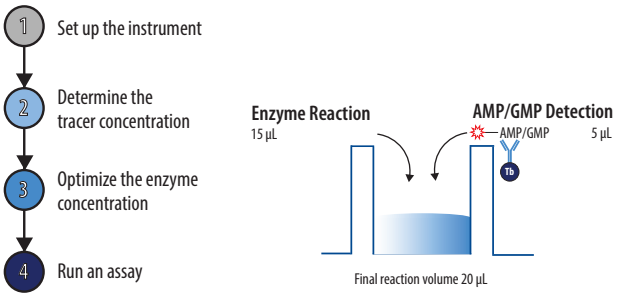



**Figure 2. An outline of the procedure.** The assay consists of 4 main steps with a mix-and-read format.

### 4.1 Set Up the Instrument

Becoming familiar with ideal instrument settings for TR-FRET is essential to the success of the Transcreener® AMP²/GMP² TR-FRET Red Assay.

**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.

#### 4.1.1 Verify That the Instrument Measures TR-FRET

Ensure that the instrument is capable of measuring TR-FRET (not simply fluorescence intensity) of the terbium:HiLyte647 TR-FRET pair ($Ex_{320}/Em_{615}/Em_{665}$).

#### 4.1.2 Define the Maximum TR-FRET Window for the Instrument

Measuring high (0% ATP/cAMP/cGMP conversion) and low (100% ATP/cAMP/cGMP conversion) FRET will define the maximum assay window of your specific instrument. Prepare High and Low FRET Mixtures in quantities sufficient to perform at least 6 replicates for each condition.

Use ATP/cAMP/cGMP at 0.75X and AMP/GMP HiLyte647 Tracer at 0.25X concentration in a 20 µL final reaction volume. This mimics the 2-fold dilution when adding an equal volume of detection mixture to an enzyme reaction. As an example, the 1X detection mixture may contain 10 µM ATP/cAMP/cGMP. After adding this to the enzyme reaction, the concentration in the final 20 µL reaction volume would be 7.5 µM.

***High FRET Mixture***

Prepare the following solution:

| Component | Stock Concentration | Final Concentration | Example: 25 Assays | Your Numbers |
|---|---|---|---|---|
| AMP²/GMP² Antibody-Tb | 800 nM | 3 nM | 1.9 µL | |
| Tris Solution | 1 M | 18.75 mM | 9.4 µL | |
| AMP/GMP HiLyte647 Tracer | 10 µM | 15 nM | 0.8 µL | |
| ATP/cAMP/cGMP | 5 mM | 7.5 µM | 0.8 µL | |
| Water | | | 487.3 µL | |
| **Total** | | | **500.0 µL** | |

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

***Low FRET Mixture***

Prepare the following solution:

| Component | Stock Concentration | Final Concentration | Example: 25 Assays | Your Numbers |
|---|---|---|---|---|
| AMP²/GMP² Antibody-Tb | 800 nM | 3 nM | 1.9 µL | |
| Tris Solution | 1 M | 18.75 mM | 9.4 µL | |
| AMP/GMP HiLyte647 Tracer | 10 µM | 15 nM | 0.8 µL | |
| AMP/GMP | 5 mM | 7.5 µM | 0.8 µL | |
| Water | | | 487.3 µL | |
| **Total** | | | **500.0 µL** | |

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

### 4.1.3 Measure the TR-FRET

Test the Z′ factor and assay window on your instrument by adding 20 µL of the Low FRET Mixture in 16 wells and 20 µL of High FRET Mixture in 16 wells. Calculate the Z′ factor using the equation below; values greater than 0.7 are acceptable.

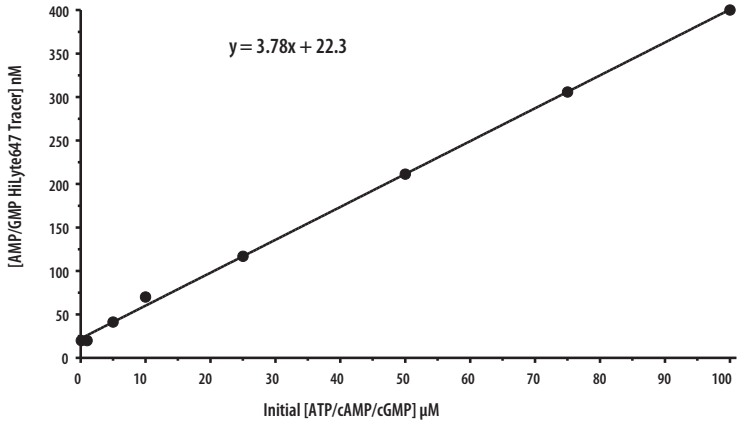
**Caution:** Contact BellBrook Labs Technical Service for assistance if the calculated Z′ factor is less than 0.7.

$$Z' = 1 - \frac{[(3 \times SD_{\text{High FRET Mixture}}) + (3 \times SD_{\text{Low FRET Mixture}})]}{|(\text{mean of High FRET Mixture ratio 665:615}) - (\text{mean of Low FRET Mixture ratio 665:615})|}$$

## 4.2 Determine the AMP/GMP HiLyte647 Tracer Concentration

The Transcreener® AMP²/GMP² TR-FRET Red Assay requires detection of AMP/GMP in the presence of excess ATP/cAMP/cGMP (assuming initial velocity enzyme reaction conditions) using an antibody with a finite selectivity for the monophosphate vs. the triphosphate or cyclic monophosphate. The concentration of AMP/GMP HiLyte647 tracer determines the total assay window and the AMP/GMP detection range; the amount needed primarily depends upon the initial ATP/cAMP/cGMP concentration in the enzyme reaction.

**Figure 3. Linear relationship between [ATP/cAMP/cGMP] and [AMP/GMP Tracer].** The tracer concentration can be calculated using the equation: **y = 3.78x + 22.3**




### 4.2.1 Calculating the Tracer Amount

As shown in **Figure 3**, the relationship between ATP/cAMP/cGMP and AMP/GMP HiLyte647 Tracer concentrations is linear. (Though shown for up to 100 µM ATP/cAMP/cGMP, the relationship is valid to 1,000 µM.) Therefore, the quantity of AMP/GMP HiLyte647 Tracer for enzyme reactions that use between 0.1 µM and 1,000 µM initial ATP/cAMP/cGMP can be determined using the equation **y = mx + b**, where **x** = initial [ATP/cAMP/cGMP] (µM) in the 15 µL enzyme reaction, **y** = [AMP/GMP HiLyte647 Tracer] (nM) in the 5 µL of 1X AMP/GMP Detection Mixture, **m** (slope) = 3.78, and **b** (y-intercept) = 22.3. We recommend a final reaction volume of 20 µL.

For example, if you are using 3 µM ATP/cAMP/cGMP in a 15 µL enzyme reaction, the optimal AMP/GMP HiLyte647 Tracer concentration in the 1X GDP Detection Mixture (assuming 5 µL of AMP/GMP Detection Mixture was added to each 15 µL enzyme reaction) would be (3.78 × 3) + 22.3 = 33.6 nM.

### 4.2.2 Optimizing the Tracer Concentration

Using the AMP/GMP HiLyte647 Tracer concentration calculated using the equation in **Figure 3** will produce excellent results for most users. If it does not produce the results you require, simply optimize the tracer concentration in a stepwise fashion using the AMP/GMP HiLyte647 Tracer concentration (X) from the line as a starting point. Try performing a standard curve (see **Section 7.1**) at 0.5 × [Y], [Y], and 1.5 × [Y] tracer concentrations to find an assay window that suits your needs. See **Section 6** for troubleshooting suggestions.

## 4.3 Optimize the Enzyme Concentration

Perform an enzyme titration to identify the optimal enzyme concentration for the Transcreener® AMP²/GMP² TR-FRET Red Assay. Use enzyme buffer conditions, substrate, and ATP/cAMP/cGMP concentrations that are optimal for your target enzyme and AMP/GMP HiLyte647 Tracer concentration calculated as described in **Section 4.2**. If a compound screen is planned, you should include the library solvent at its final assay concentration. Run your enzymatic reaction at its requisite temperature and time period. Refer to **Section 7.2** 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 FRET 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:

$$EC_{80} = (80 \div (100 - 80))^{(1 \div \text{hillslope})} \times EC_{50}$$

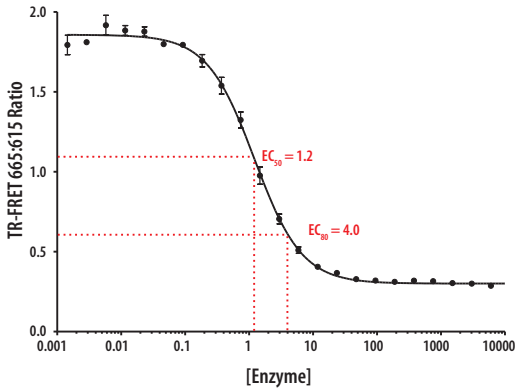



**Figure 4. Enzyme titration curve.** The ideal range of enzyme concentrations is shown in red.

### 4.3.2 Enzyme Assay Controls

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

| Component | Notes |
|---|---|
| 0% ATP/cAMP/cGMP Conversion Control | This control consists of the AMP/GMP Detection Mixture, the enzyme reaction components (without enzyme), and 100% ATP/cAMP/cGMP (0% AMP/GMP). It defines the upper limit of the assay window. |
| 100% ATP/cAMP/cGMP Conversion Control | This control consists of the AMP/GMP Detection Mixture, the enzyme reaction components (without enzyme), and 100% AMP/GMP (0% ATP/cAMP/cGMP). 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/cAMP/cGMP). |
| AMP/GMP Standard Curve | Although optional, an AMP/GMP 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 Tris Solution. |

## 4.4 Run an Assay

1. Add the enzyme reaction mixture to test compounds and mix on a plate shaker.
2. Start the reaction by adding ATP/cAMP/cGMP, then mix. The final volume of the enzyme reaction mixture should be 15 µL. Incubate at a temperature and time ideal for the enzyme target before adding the AMP/GMP Detection Mixture.
3. Prepare 1X GDP Detection Mixture as follows:

| | ATP Concentration: Examples | | | |
|---|---|---|---|---|
| **Component** | **1 µM** | **10 µM** | **100 µM** | **Your Numbers** |
| AMP²/GMP² Antibody-Tb | 100 µL | 100 µL | 100 µL | |
| AMP/GMP HiLyte647 Tracer | 13.1 µL | 30.1 µL | 200.2 µL | |
| Tris Solution (1 M) | 125 µL | 125 µL | 125 µL | |
| Water | 4,762.0 µL | 4745.0 µL | 4574.8 µL | |
| **Total** | **5,000 µL** | **5,000 µL** | **5,000 µL** | |

Final concentrations in the detection mixture should be 16 nM GDP Antibody-Tb, 25 mM Tris Solution, and the tracer concentration calculated using the equation in Figure 3. An example is shown below:

| | y = 3.78x + 22.3 | | |
|---|---|---|---|
| ATP | 1 µM | 10 µM | 100 µM |
| AMP/GMP HiLyte647 Tracer | 26.1 nM | 60.1 nM | 400.3 nM |

4. Add 5 µL of 1X AMP/GMP Detection Mixture to 15 µL of the enzyme reaction. Mix using a plate shaker.
5. Incubate at room temperature (20–25°C) for at least 2 hours and measure TR-FRET.

# 5.0 General Considerations

## 5.1 Assay Types

### 5.1.1. Endpoint Assay

The Transcreener® AMP²/GMP² TR-FRET Red Assay is designed for endpoint readout.

### 5.1.2 Real-Time Assay

This assay can be performed in real time by eliminating stop reagents and including the AMP/GMP Detection Mixture components (antibody and tracer) in the enzyme reaction. However, this mode should only be used for relative activity comparisons, because the extended signal equilibration time precludes accurate quantitation of AMP/GMP. A standard curve run under similar conditions (continuous mode) will help in extrapolating the FRET ratios to amount of AMP or GMP product formed.

## 5.2 Reagent and Signal Stability

The Transcreener® technology provides a robust and stable assay method to detect AMP/GMP.

### 5.2.1 Signal Stability

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

### 5.2.2 AMP/GMP Detection Mixture Stability

It is important that the AMP/GMP Detection Mixture is prepared just prior to addition to the enzyme reaction. If you prepare the AMP/GMP Detection Mixture more than 30 minutes before addition, store it on ice or at 4°C until needed to help decrease the equilibration time.

### 5.2.3 Stopping the Reaction

We have inhibited the activity of several phosphodiesterases and ubiquitin ligases by the addition of Stop Buffer B (200 mM HEPES, 0.2% Brij®-35, and 400 mM EDTA [pH 7.5]), by quenching $MgCl_2$ with EDTA. This buffer can be purchased separately: Part #2027 (1 mL) or 2032 (10 mL)

## 6.0 Troubleshooting

| Problem | Possible Causes and Solutions |
|---|---|
| Low selectivity | *Suboptimal tracer concentration*<br>• To achieve maximum sensitivity and assay window, the AMP/GMP tracer concentration must be optimized for each starting ATP/cAMP/cGMP concentration.<br>*ATP/cAMP/cGMP concentration out of range*<br>• Ensure that the starting ATP/cAMP/cGMP concentration is in the range of 1–1,000 µM. |
| No change in TR-FRET observed | *Low antibody/tracer activity*<br>• 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.<br>*Interference from metal ions*<br>• $Mn^{2+}$ or heavy metals like $Cu^{2+}$, $Fe^{2+}$, $Fe^{3+}$, $Cr^{3+}$, or $Co^{2+}$ can quench terbium at higher concentrations. This effect can be relieved by using an EDTA-containing stop buffer (see **Section 5.2.3**). Use a minimum molar ratio of at least 4X EDTA to metal ions. |
| High background signal or change in signal after incubation | *Nonspecific ATP/cAMP/cGMP hydrolysis*<br>• Aliquot the stock solution of nucleotide or prepare it fresh each time to avoid frequent freeze-thaw cycles.<br>*Interference from impurities*<br>• Nuclease contamination in the buffer can cause the assay window to collapse, causing a change in FRET ratio. We recommend using nuclease-free water and freshly prepared buffer for each assay.<br>• Some compounds may interfere with the detection mixture, causing a change in signal (see **Section 7.2**).<br>• Bovine serum albumin (BSA) at concentrations >1% interferes with the detection reagents. Detergents, such as Brij-35, can be substituted for BSA in the enzyme reaction to prevent nonspecific binding of enzymes and substrates to the plate. |

## 7.0 Appendix

### 7.1 Standard Curves

A standard curve (**Figure 5**) is required to convert FRET ratios to product formation (AMP or GMP) for quantitative data analysis. Because the Transcreener® AMP²/GMP² TR-FRET Red Assay relies on a competitive binding reaction, the response is nonlinear, and therefore the signal is not directly proportional to reaction progress.

The wells for the standard curve should contain all AMP/GMP reaction components except the enzyme and receive AMP/GMP Detection Mixture. The curve is constructed to mimic an enzyme reaction: starting at the ATP/cAMP/cGMP concentration used for the screening reactions, ATP/cAMP/cGMP is decreased in increments and the AMP/GMP concentration is increased proportionally, keeping the sum of their concentrations [ATP/cAMP/cGMP + AMP/GMP] constant.

We recommend using a 12-point curve with concentrations of ATP/cAMP/cGMP and AMP/GMP corresponding to 0%, 0.5%, 1%, 2%, 3%, 5%, 7.5%, 10%, 15%, 30%, 50%, and 100% conversion (see **Table 1**). Allow 2-3 hours incubation prior to FRET measurement for complete equilibration.

| % Conv. | ATP (µM) | AMP (µM) |
|---|---|---|
| 100 | 0 | 100 |
| 50 | 50 | 50 |
| 30 | 70 | 30 |
| 15 | 85 | 15 |
| 10 | 90 | 10 |
| 7.5 | 92.5 | 7.5 |
| 5 | 95 | 5 |
| 3 | 97 | 3 |
| 2 | 98 | 2 |
| 1 | 99 | 1 |
| 0.5 | 99.5 | 0.5 |
| 0 | 100 | 0 |

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

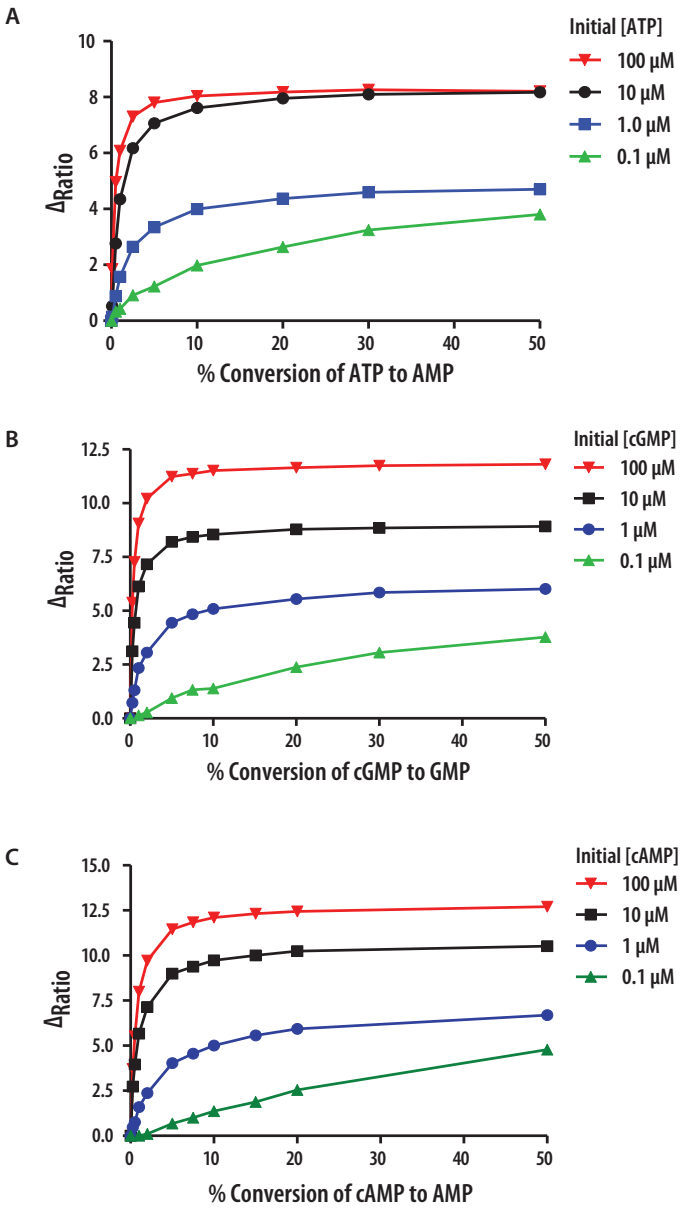



**Figure 5. Standard curves.** Data are shown for ATP to AMP (**A**), cGMP to GMP (**B**), and cAMP to AMP (**C**) standard curves for initial substrate concentrations of 0.1–100 µM in the15 µL mock enzyme reaction. All assays were performed in 384-well plates (n = 12) and read on the Perkin Elmer EnVision multimode plate reader. $\Delta_{Ratio}$ is the change in TR-FRET 665:615 ratio from 0%. **D**) Z′ values for initial velocity detection (10% conversion for 1 µM, 10 µM and 100 µM ATP/ADP standard curves, 30% for 0.1 µM) and lower limits of detection (LLD). LLD = the concentration of AMP/GMP that generates Z′ >0.

**D**

| | 0.1 µM Std Curve | | 1.0 µM Std Curve | | 10 µM Std Curve | | 100 µM Std Curve | |
|---|---|---|---|---|---|---|---|---|
| | Z′ at 30% Conversion | LLD (nM) | Z′ at 10% Conversion | LLD (nM) | Z′ at 10% Conversion | LLD (nM) | Z′ at 10% Conversion | LLD (nM) |
| ATP:AMP | 0.8 | 5 | 0.9 | 5 | 0.9 | 50 | 0.9 | 250 |
| cGMP:GMP | 0.7 | 7.5 | 0.7 | 10 | 0.8 | 50 | 0.9 | 250 |
| cAMP:AMP | 0.7 | 7.5 | 0.7 | 10 | 0.8 | 50 | 0.9 | 250 |

Use the following equations to calculate the Z' factor:

$$\Delta ratio = ratio_{initial\ [GTP]} - ratio_{sample}$$

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

## 7.2 Summary of Additive Effects on the Transcreener® AMP²/GMP² TR-FRET Assay

The assay window at 10% substrate conversion 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) | 5-Hour Tolerance (0–10% Conversion Signal) |
|---|---|---|
| **Solvents** | | |
| Acetonitrile | >50% | 12.5% |
| DMSO | 3.13% | 3.13% |
| Ethanol | >50% | 12.50% |
| Methanol | >50% | 25.00% |
| Glycerol | >50% | 0.39% |
| **Detergents** | | |
| Brij-35 | 0.94% | 0.47% |
| CHAPS | 1.25% | 0.08% |
| NP40 | 0.16% | 0.16% |
| SDS | 0.04% | 0.04% |
| Triton X-100 | 0.31% | 0.31% |
| Sodium deoxycholate | 0.00% | 0.16% |
| N-lauroyl sarcosine | 0.08% | 0.04% |
| **Metal chelates** | | |
| EDTA | 125 mM | 62.5 mM |
| EGTA | >250 mM | 125 mM |
| **Reductants** | | |
| Beta mercaptoethanol | 6.25% | 0.02% |
| Dithiothreitol | >500 mM | 0.031 mM |

| Component | 5-Hour Tolerance (0–100% Conversion Signal) | 5-Hour Tolerance (0–10% Conversion Signal) |
|---|---|---|
| **Salts** | | |
| Ammonium acetate | 125 mM | 125 mM |
| Ammonium sulfate | 3.91 mM | 3.91 mM |
| Calcium chloride | 15.62 mM | 7.81 mM |
| Magnesium acetate | 15.62 mM | 3.91 mM |
| Magnesium chloride | 15.62 mM | 7.81 mM |
| Magnesium sulfate | 1.95 mM | 1.95 mM |
| Manganese chloride | 15.62 mM | 7.81 mM |
| Potassium chloride | 500 mM | 125 mM |
| Sodium azide | 1.25% | 1.25% |
| Sodium bromide | 12.5 mM | 3.12 mM |
| Sodium chloride | 12.5 mM | 6.25 mM |
| **Phosphatase Inhibitors** | | |
| Glycerol phosphate | 15.62 mM | 7.81 mM |
| Imidazole | 62.5 mM | 62.5 mM |
| Sodium fluoride | 7.81 mM | 3.91 mM |
| Sodium molybdate | 31.25 mM | 31.25 mM |
| Sodium tartrate | >400 mM | 200 mM |
| Sodium orthovanadate | 7.81 mM | 7.81 mM |
| Sodium pyrophosphate | 0.39 mM | 0.78 mM |
| **Carrier Proteins/Coactivators** | | |
| BSA | >1.0 mg/mL | >1.0 mg/mL |
| BGG | >2.5 mg/mL | >2.5 mg/mL |

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

## 8.0 Bibliography

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