Optimizing Settings to Validate the BMG LABTECH CLARIOstar HTS Microplate readers with the Transcreener TR-FRET Assays.

Meera Kumar[1] and Carl Peters, Ph.D,[2]
[1]BellBrook Labs, Madison-WI, [2] BMG Labtech, Durham,NC,USA

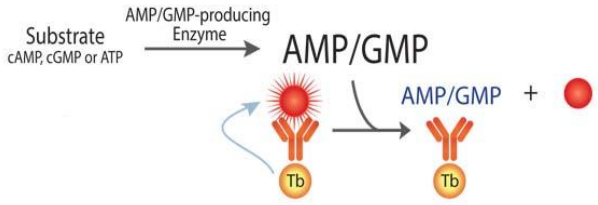
Introduction

This application protocol describes the optimal instrument parameters used to validate the CLARIOstar® Microplate Reader with the following assays from BellBrook Labs:

- Transcreener ADP^2 TR-FRET (3011)
- Transcreener AMP^2/GMP^2 TR-FRET (3020)

> Transcreener® is a universal, high throughput biochemical assay platform based on the detection of nucleotides, which are formed by thousands of cellular enzymes. Many of these enzymes catalyze the covalent regulatory reactions that are central to cell signaling; e.g., phosphorylation, methylation, and are of high interest as therapeutic targets.

The Transcreener® TR-FRET Assays are a single step, competitive immunoassay for direct detection of nucleotides with a far red time-resolved Förster-resonance-energy-transfer (TR-FRET) readout. The reagents for all of the assays are a far red Tracer bound to a highly-specific monoclonal antibody-Terbium conjugate. Excitation of the Terbium complex in the UV range (ca. 330 nm) results in energy transfer to the Tracer and emission at a higher wavelength (665 nm) after a time delay. Nucleotide diphosphate or monophosphate produced by the target enzyme displaces the tracer from the antibody, leading to a decrease in TR-FRET (Figure 1). The use of a red tracer minimizes interference from fluorescent compounds and light scattering. The Transcreener® TR-FRET Assays are designed specifically for HTS with a single addition, mix-and-read format.



Validation criteria

A critical factor in realizing the advantages of the Transcreener HTS assays is the correct setup of the microplate reader used for data acquisition. Proper selection of instrument settings have a profound impact on the sensitivity of the assays. The key instrument parameters for Transcreener HTS assay performance were determined by running a standard curve for conversion of 10 µM ATP to ADP, mimicking a typical kinase enzyme reaction. Starting with 10 µM ATP, ADP was added in increasing amounts and ATP was decreased proportionately, maintaining a total adenine nucleotide concentration of 10 µM. The integration times were varied to determine the requirements for a Z' > 0.5. Validation of an instrument for use with the Transcreener TR-FRET Assays requires a Z' of at least 0.7 at 10% conversion of 10 µM ATP (1 µM ADP/9 µM ATP).

Clariostar

- High performance, modular, and upgradable instrument that performs all of the leading non-isotopic detection technologies
- Assay flexibility is given by Triple Detection Technology: Advanced LVF MonochromatorsTM, spectrometer, and filters
- LVF MonochromatorsTM have filter-like sensitivity and flexibility

- An integrated fluorophore library contains spectra for the most common fluorophores while offering recommended settings

Materials

- **ATP/ADP Mixture** - 4 mM $MgCl_2$, 2 mM EGTA, 50 mM HEPES(pH 7.5), 1% DMSO, 0.01% Brij-35, and ATP/ADP (combined to a constant adenine concentration of 10 µM)
- **ADP Detection Mixture -** 1X Stop & Detect Buffer C, 8 nM ADP[2] Antibody-Tb, and 27 nM ADP HiLyte647 Tracer.
- **High FRET Mixture**- 8nM ADP[2] Antibody-Tb, 27 nM ADP HiLyte647 Tracer, 10 µM ATP in 1X Stop & Detect Buffer C.
- **Low FRET Mixture -** 8 nM ADP[2] Antibody-Tb, 27 nM ADP HiLyte647 Tracer, 10 µM ADP in 1X Stop & Detect Buffer C.

For a detailed procedure on how to prepare a standard curve, please refer to the appropriate Transcreener Technical Manual (http://www.bellbrooklabs.com/transcreener_hts_assays.html).

Methods

Assay preparation

1. Dispense 10 µL of each ATP/ADP combination across an entire row of a 384-well plate.
2. Add 10 µL of ADP Detection Mix to those rows.
3. Dispense 10 µL of the 10 µM ATP/0 µM ADP combination into row P.
4. Dispense 10 µL of high FRET mixture into wells P1-P12.
5. Dispense 10 µL of low FRET mixture into wells P13-P24.

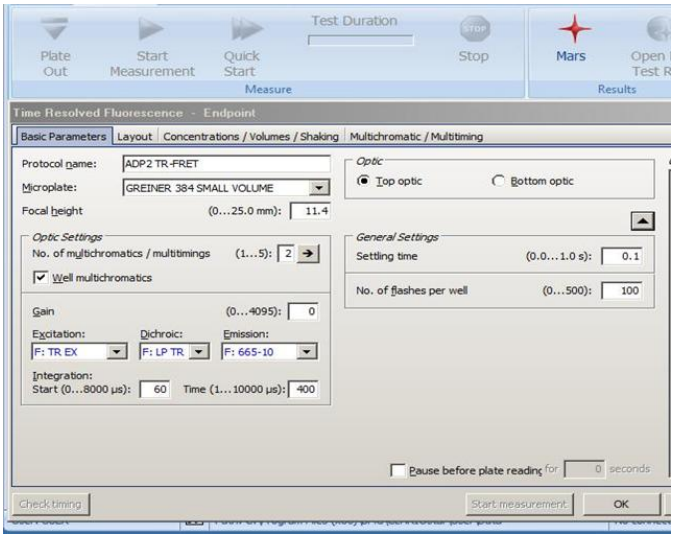
Instrument setup

Set up the CLARIOstar Reader with the settings in Table 1.

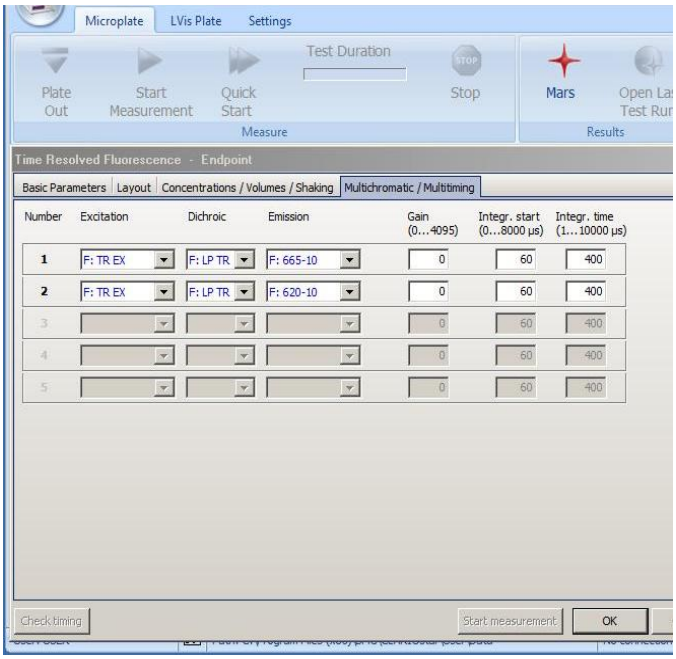
Table 1. Recommended CLARIOstar Instrument Settings

Parameter	Setting
Optical Filter Set	Transcreener TR-FRET CLARIOstar Specific Filters. EXC-TR-EX Dichroic: LP-TR EMS 1: 665 nm/EMS 2: 620 nm
Integration start and time	60/400 µs
Gain	2400
Positioning Delay	0.1 sec
Flash Number	variable

Install the Transcreener TR-FRET filters in the CLARIOstar and make sure that the filters are in the filter table. In the Basic Parameters page make sure the "No. of multichromatics / multimings" is 2 and the box next to "Well multichromatics" is checked



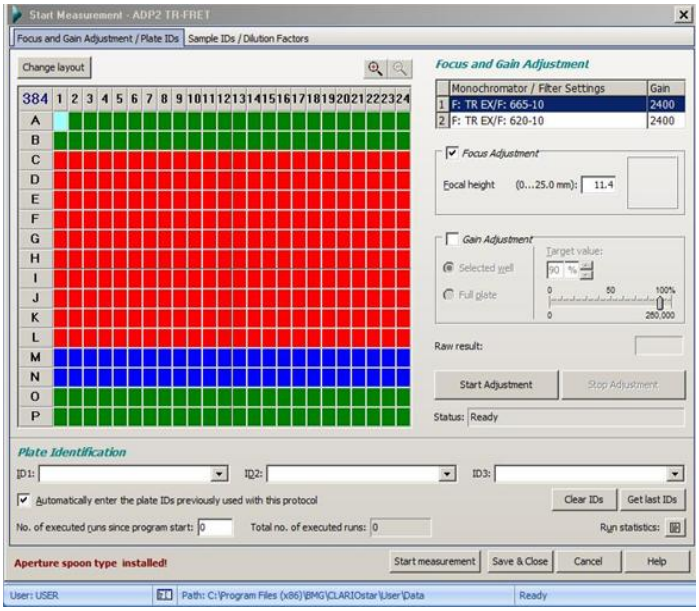
In the Multichromatic/Multitiming page select the filter and dichroics from the Transcreener TR-FRET filter set.



Proceed with the following steps to optimize z-height focus

1. Select the **'Focus and Gain Adjustment / Plate IDs'** tab from the **Measurement** scree
2. For optimization purposes, select a well containing **Low FRET** from the plate layout
3. Select **'Focus Adjustment'**
4. Select **'Start Adjustment'** to begin the optimization process

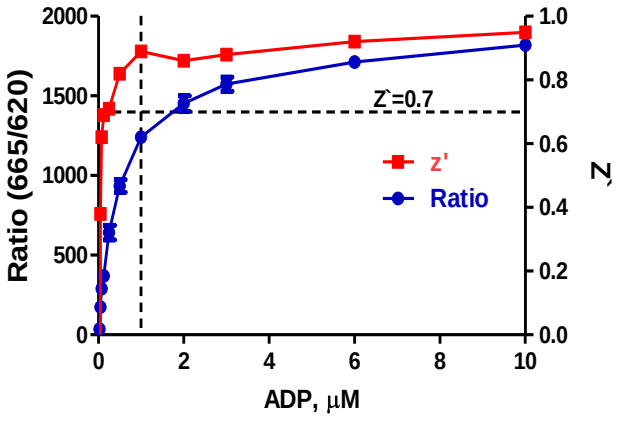
The same measurement setting can be used for subsequent plates as long as the volumes, tracer and concentrations remain the same.



Results

Sample TR-FRET standard curve

As the ratio of ADP:ATP increases, the proportion of bound tracer vs. free tracer decreases, resulting in an overall decrease in FRET. Assay plates containing the 15-point standard curve were read on the Clariostar HTS Microplate Reader.



A: Z' and ratios observed in a standard curve for conversion of 10 µM ATP to ADP. **B**: Zoomed view of the 0-3 µM ADP section of the standard curve, with dashed lines indicating that the observed Z' and ratios exceed the validation requirements.

Conclusions

This application protocol demonstrates the validation of the BMG LABtech`s CLARIOstar for use with the Transcreener TR-FRET Assays. By utilizing the optimized instrument settings suggested here, Z' values > 0.7 is achievable with short read times.

Additional Information

Please visit www.bellbrooklabs.com or contact BellBrook Labs for pricing for the Transcreener® Assays. Custom quotes are available for bulk orders.

Ordering Information

Phone Orders:
608.443.2400
866.3137881

Fax Orders:
608.441.2967

Email Orders:
info@bellbrooklabs.com

Technical Information

For technical information, please contact:

Meera Kumar, Senior Applications Scientist
Tel: 608.443.2400
Toll-Free: 866.313.7881
Email: meera.kumar@bellbrooklabs.com.

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