Application Note

VALIDATION OF THE MITHRAS² LB 943 MONOCHROMATOR MULTIMODE READER WITH THE TRANSCREENER® GDP TR-FRET RED ASSAY

High-performance time-resolved FRET analysis

Abstract

The activity of enzymes that convert guanosine triphosphate (GTP) to its diphosphate can be determined by measuring the concentration of the produced guanosine diphosphate (GDP). BellBrook Labs has developed the Transcreener® GDP TR-FRET Red Assay, where a TR-FRET signal is measured that is proportional to the amount of generated GDP. For the detection of the fluorescence signal, a suitable plate reader is required, such as the Mithras² LB 943 developed by Berthold Technologies. In order to confirm the compatibility of the Transcreener® GDP TR-FRET Red Assay with the Mithras² LB 943, we have determined a GDP/GTP standard curve that mimics an enzyme reaction. The suitably large assay window, low standard errors and the resulting robust standard curve with a Z' value of more than 0.8 at 10 % conversion of GTP confirm that the Mithras² LB 943 is a suitable device for measuring the Transcreener® GDP TR-FRET Red Assay.

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Introduction

Fluorescence/Förster-resonance-energy-transfer (FRET) is a technique which is widely used in biomedical and pharmaceutical research. Using a time-resolved approach facilitates high-throughput-screenings of test compounds, as these compounds might be fluorescent themselves and thus could cause interferences in a classical FRET experiment.

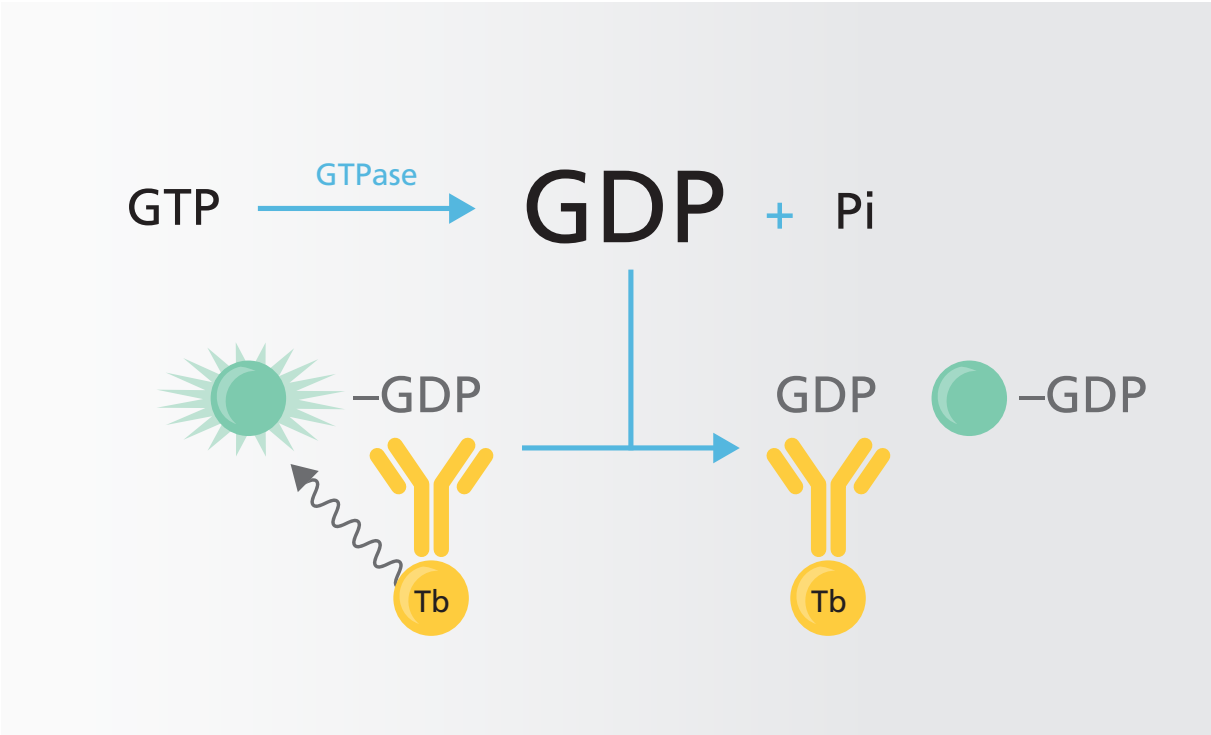


Figure 1: Basic principle underlying the Transcreener® GDP TR-FRET Red Assay (from www.bellbrooklabs.com)

THE BELLBROOK LABS TRANSCREENER® FLUORESCENCE INTENSITY ASSAY

The Transcreener® Fluorescence Intensity Assays are single step, competitive immunoassays for direct detection of nucleotides with a red fluorescence intensity readout. The reagents for all of the assays are a red tracer bound to a highly-specific monoclonal antibody-quencher conjugate. The nucleotide diphosphate or monophosphate produced by the target enzyme displaces the tracer from the antibody-quencher conjugate, resulting in an increase in fluorescence intensity (Figure 1). The use of a red tracer minimizes interference from fluorescent compounds and light scattering. The Transcreener® FI Assays are designed specifically for HTS with a single addition, mix-and-read format.

Mithras² LB 943
Monochromator Multimode Reader

The high-end performance multimode reader

The Mithras² LB 943 is a high-end microplate multimode reader based on monochromator technology with excellent performance. Characterized by its sensitivity and robustness, especially in luminescence and BRET measurements, the reader supports all important reading technologies:

- Luminescence
- BRET and BRET²
- Fluorescence
- FRET
- Fluorescence Polarisation
- UV/VIS absorbance
- AlphaScreen® and AlphaLISA®
- Time-resolved fluorescence
- TR-FRET

MITHRAS² ADDITIONAL FEATURES

- Monochromator & filter technology
- Detectors: 2 low-noise PMTs (up to 850 nm) and 1 ultra-low noise PMT operated in single photon counting mode, photo diode (200 – 1000 nm)
- Top & bottom reading
- Automatic Plate height Adjustment (Z optimization)
- Up to 4 JET injectors (98% accuracy & precision over entire volume range)
- All microplate formats up to 1536-well
- Shaking and incubation up to 45°C

Different reading technologies have their own demands on the optical system for optimal performance. In contrast to conventional multi-technology instruments, the Mithras multimode microplate reader has been designed with a proprietary optical system consisting of separate optical paths for different reading technologies (mDOPS). The separated light paths of the mDOPS optical system ensure that the needs for high sensitivity and a wide dynamic range are met for each reading technology. This results in high-end performance that typically can be achieved with dedicated instruments only.

Materials and Settings

MATERIALS

- Berthold Technologies Mithras² LB 943 Monochromator Multimode Reader
- Transcreener® GDP TR-FRET Red Assay Kit (Catalogue No. 3021)
- White, small-volume 384-well microplate (Greiner 784075)

INSTRUMENT SETTINGS

- Excitation filter: ID-number 54083-01
- Emission filter donor: ID-number 47731-01
- Emission filter acceptor: ID-number 60729-01

Assay Window

In order to define the maximum assay window for the Mithras² LB 943 plate reader, we measured the TR-FRET signal of the low FRET (10 µM GTP = 0% GTP conversion) and high FRET (10 µM GDP = 100% GTP conversion) controls, as described in the technical manual. The mean signals of 24 wells for each control were as follows in Table 1.

Sample	Donor		Acceptor		**FRET ratio**	
	Mean RFU	SD	Mean RFU	SD	**Mean**	SD
High control	80315	2749	5035	241	**0.0627**	0.0014
Low control	53668	1869	19559	961	**0.3644**	0.0103

Table 1: Determination of the assay window. RFU = relative fluorescence units

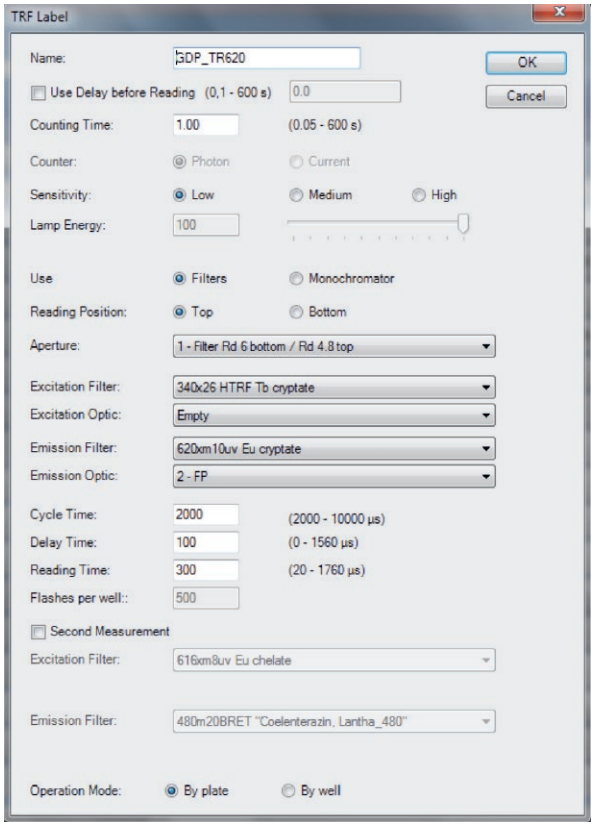


Figure 2: Screenshot of the instrument settings dialogue (donor emission) in the MikroWin 2010 software

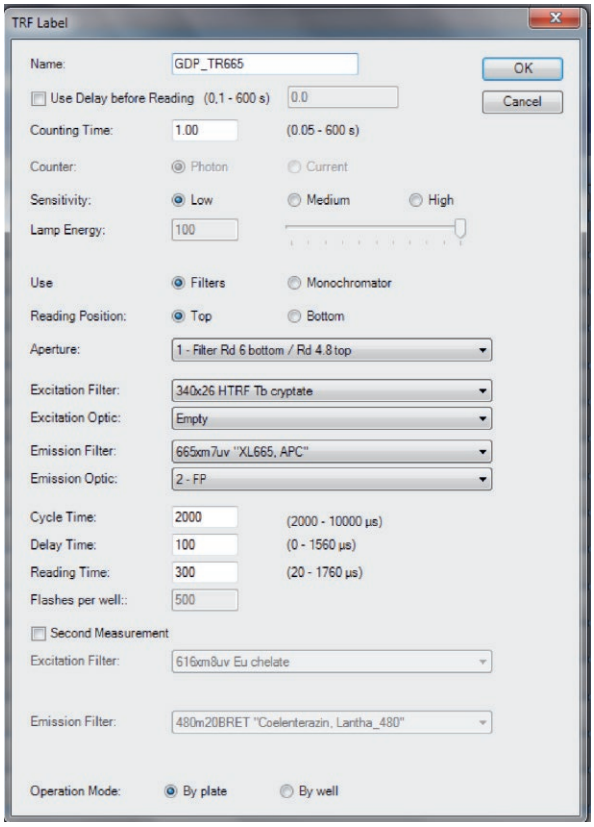


Figure 3: Screenshot of the instrument settings dialogue (acceptor emission) in the MikroWin 2010 software

GDP/GTP Standard Curve

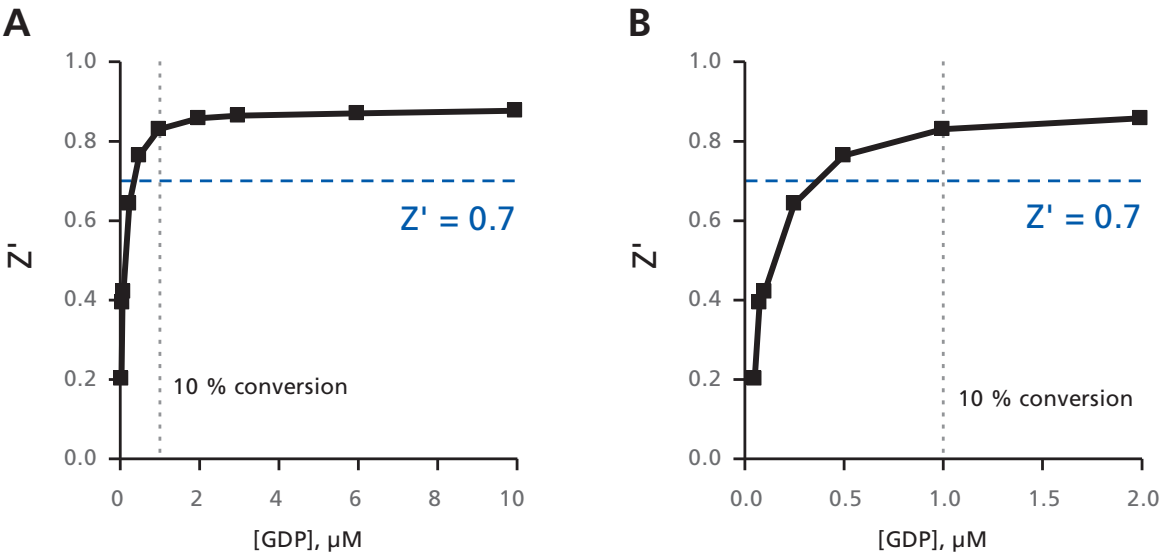
During an enzyme reaction, GTP would be converted to GDP. This reaction was mimicked by assessing different ratios of GDP and GTP, starting from 10 µM GTP/0 µM GDP to 0 µM GTP/10 µM GDP. The concentration of guanine nucleotides thereby remains constant at 10 µM. As the concentration of GDP increases, more of the tracer bound to the antibody will be displaced and TR-FRET will be reduced. The assay components were as follows:

- GTP/GDP mixture (combined to a constant guanine nucleotide concentration of 10 µM).
- GDP detection mixture: 1X Stop & Detect Buffer C, 26.8 nM GDP HiLyte647 Tracer, and 4 nM GDP Antibody-Terbium Conjugate.

10 µl of GDP detection mixture was given to 10 µl of GTP/GDP solutions of different GTP/GDP ratios. Thus, a final volume of 20 µl was present in each well of a white, small-volume 384-well plate and incubated for 90 min at room temperature prior to measurement.

In order to validate an instrument for use with the Transcreener® GDP TR-FRET Red Assay, a Z' ≥ 0.7 at 10 % conversion of 10 µM GTP should be obtained. On the Mithras² LB 943 plate reader, the Z' factor at 10 % conversion of 10 µM GTP was 0.83 (Figure 4). Thus, the Mithras² LB 943 plate reader is a suitable instrument for use with the Transcreener® GDP TR-FRET Red Assay.

Figure 4 (A): Z' values calculated from a standard curve mimic conversion of 10 µM GTP to GDP. (B) Enlarged view of the lower GDP concentrations. The horizontal dotted line respresents the Z' validation minimal qualification; the vertical dotted line the 10 % GTP conversion validation point.



Conclusions

A suitably large assay window and a Z' factor of 0.83 at 10 % conversion of 10 µM GTP were obtained, confirming the suitability of the Berthold Technologies Mithras² LB 943 Monochromator Multimode Reader for use with the Transcreener® GDP TR-FRET Red Assay.

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Version 11-2016