Application Note

VALIDATION OF THE TRISTAR² S LB 942 MULTIDETECTION MICROPLATE 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 TriStar² LB 942 developed by Berthold Technologies. In order to confirm the compatibility of the Transcreener® GDP TR-FRET Red Assay with the TriStar² LB 942, 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 almost 0.8 at 10% conversion of GTP confirm that the TriStar² LB 942 plate reader 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.

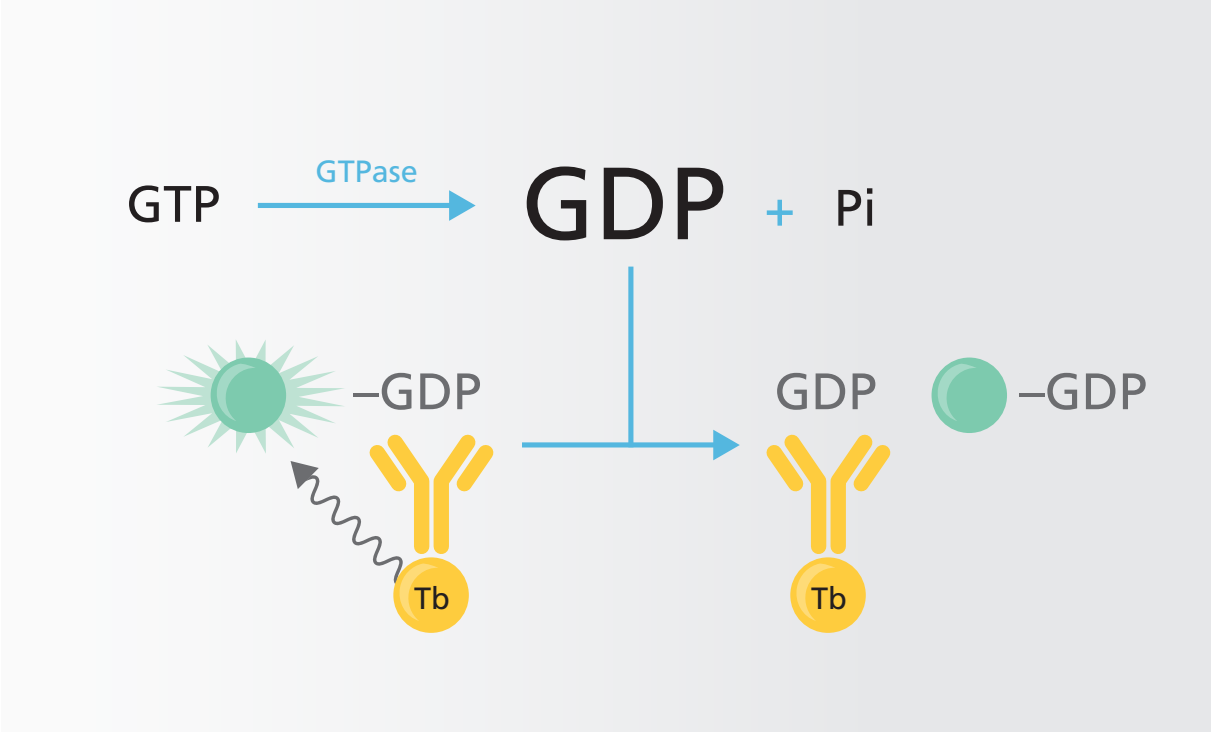


Figure 1: Basic principle underlying the Transcreener® GDP TR-FRET Red Assay (from www.bellbrooklabs.com)

THE BELLBROOK LABS TRANSCREENER® GDP TR-FRET RED ASSAY

The Transcreener® GDP TR-FRET Red Assay is a competitive immunoassay for direct detection of GDP with a far-red, TR-FRET readout. In the assay a GDP HiLyte647 tracer is used which can bind to a GDP Antibody-Tb conjugate. Upon excitation of the terbium complex with UV light an energy transfer to the antibody-bound tracer occurs after a time delay. GDP produced by the examined enzyme displaces the tracer from the antibody complex and thus reduces the TR-FRET signal (Figure 1).

TriStar² S LB 942
Multidetection Microplate Reader

Fully modular microplate reading

Developed for full modularity and equipped with the proprietary ONE-4-ALL optical system, the TriStar² S combines the user friendliness of a multimodal optical system with the sensitivity and performance of a dedicated optical device. The TriStar² S provides the flexibility for today, tomorrow, and beyond in a single system. Users can start with the reading technology they need to master their research today and upgrade when they need it. The TriStar² S series is equipped with both, top and bottom reading technology to support a wide range of detection modes, including

- Absorbance (UV/VIS)
- Fluorescence Intensity (including FRET)
- Fluorescence Polarization
- Luminescence (including BRET and BRET²)
- Time Resolved Fluorescence (TRF)
- Time Resolved FRET (TR-FRET / HTRF®).

Up to 3 proprietary JET injectors can be installed to dispense multiple activators or detection reagents at any time with high accuracy and precision as well as excellent mixing performance. The option to inject in the measurement position offers highest sensitivity for ultra-fast flash luminescence assays. A temperature controlled microplate compartment ensures stable conditions whenever temperature sensitive enzymes or cells are in use. The optional gas control unit enables adjustment of both, O_2 and CO^2 gas levels, if required for cell-based applications.

Materials and Settings

MATERIALS

- Berthold Technologies TriStar² LB 942 Multidetection Microplate 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
- Emission filter ID-number 47731
- Emission filter acceptor: ID-number 60729

Assay Window

In order to define the maximum assay window for the TriStar² LB 942 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	45412	2404	3367	363	**0.0740**	0.0043
Low control	34457	1585	18680	1212	**0.5419**	0.0195

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

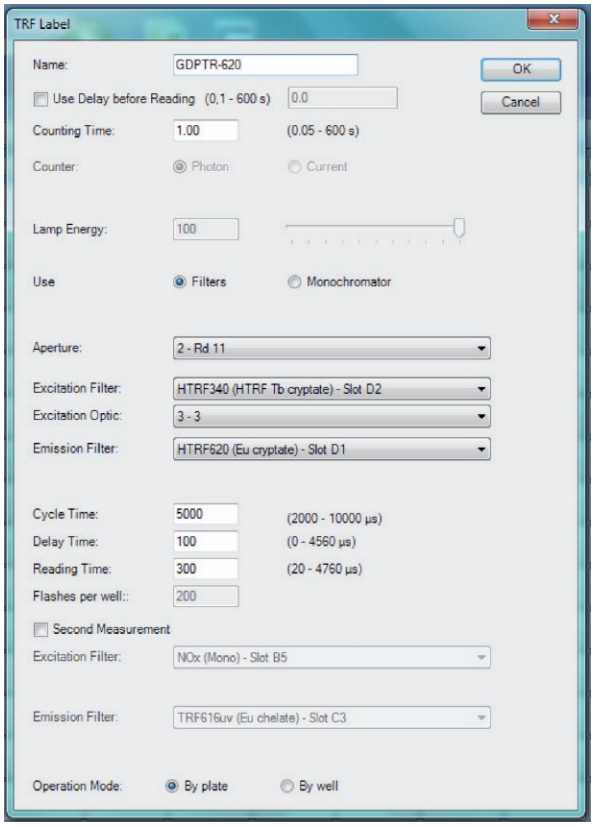


Figure 2: Screenshot of the instrument settings dialogue (donor emission) in the MikroWin 2010 software

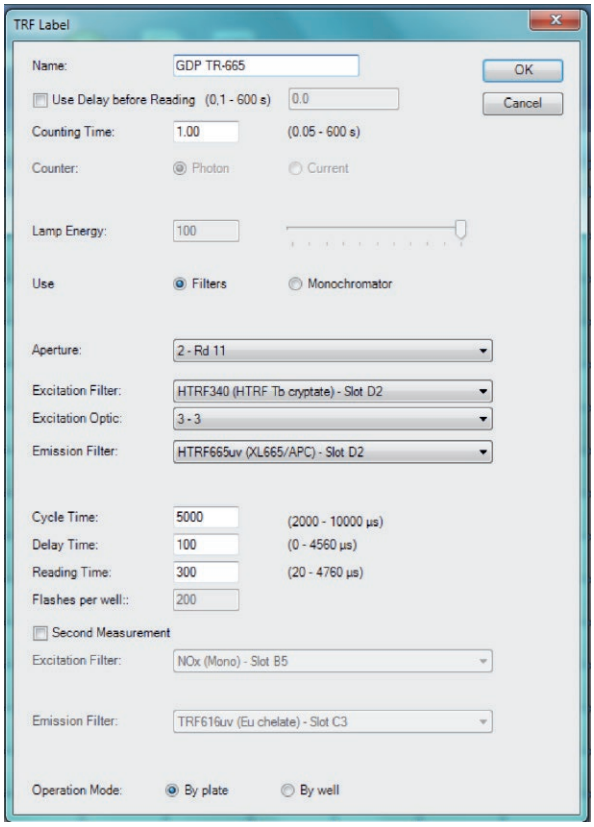


Figure 3: Screenshot of the instrument settings dialogue (acceptor emission) in the MikroWin 2010 software

GDP/GTP Standard Curve

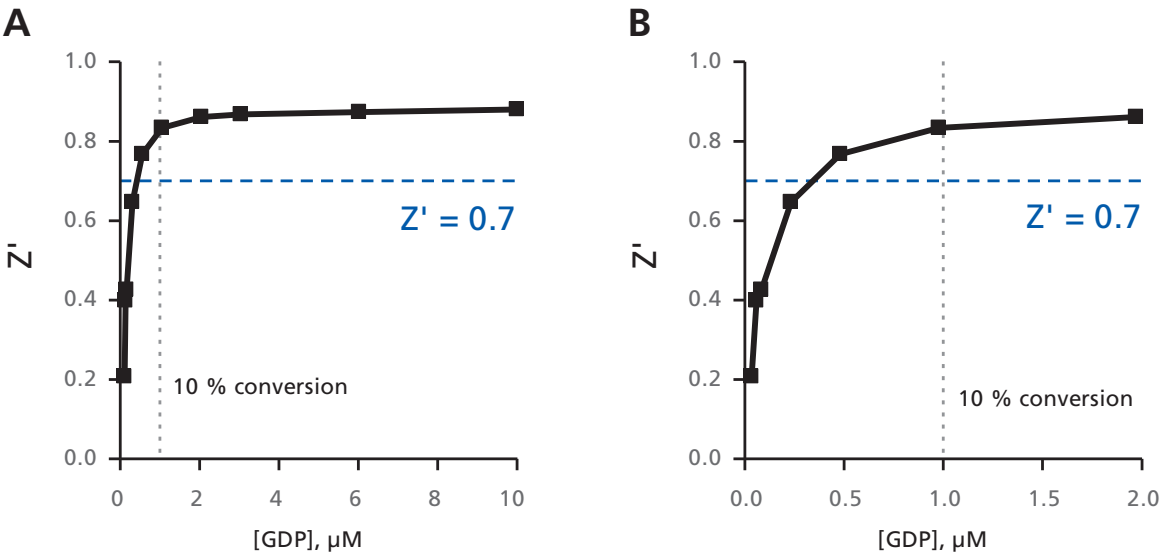
During an enzyme reaction, GTP would be converted to GDP. This reaction was mimicked by assessing different ratios of GDP and GTP, starting from 10 µM GTP/0 µM GDP to 0 µM GTP/10 µM GDP. The concentration of guanine nucleotides thereby remains constant at 10 µM. As the concentration of GDP increases, 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 TriStar² LB 942 plate reader, the Z' factor at 10% conversion of 10 µM GTP was 0.79 (Figure 4). Thus, the TriStar² LB 942 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 0.79 at 10 % conversion of 10 µM GTP were obtained, confirming the suitability of the Berthold Technologies TriStar² LB 942 Multidetection Microplate Reader for use with the Transcreener® GDP TR-FRET Red Assay.

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