Application Note

VALIDATION OF THE MITHRAS² LB 943 MONOCHROMATOR MULTIMODE READER WITH THE TRANSCREENER® FLUORESCENCE INTENSITY ASSAY

High-performance fluorescence intensity measurement

Abstract

The activity of adenosine triphosphate (ATP)-consuming enzymes can be determined by measuring the concentration of the adenosine diphosphate (ADP) released. To facilitate the assay, BellBrook Labs has developed the Transcreener® Fluorescence Intensity Assay Kit, where a fluorescence intensity signal is measured that is proportional to the amount of ADP produced. 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® Fluorescence Intensity Assay Kit with the Mithras² LB 943, we have tested the assay controls and an ADP/ATP standard curve that mimics an enzyme reaction. The very large assay window of 47 and the robust standard curve with Z' values of 0.7 or higher at ADP concentrations of at least 0.4 µM confirm that the Mithras² LB 943 is a suitable device for measuring the Transcreener® Fluorescence Intensity Assay.

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Introduction

Measurement of a fluorescence intensity signal is a common technique frequently employed for in vitro assays in drug discovery laboratories. It is a simple, versatile, and cost-effective method that has been used for several decades. A large selection of fluorescent probes is available on the market with different excitation and emission wavelengths for various needs.

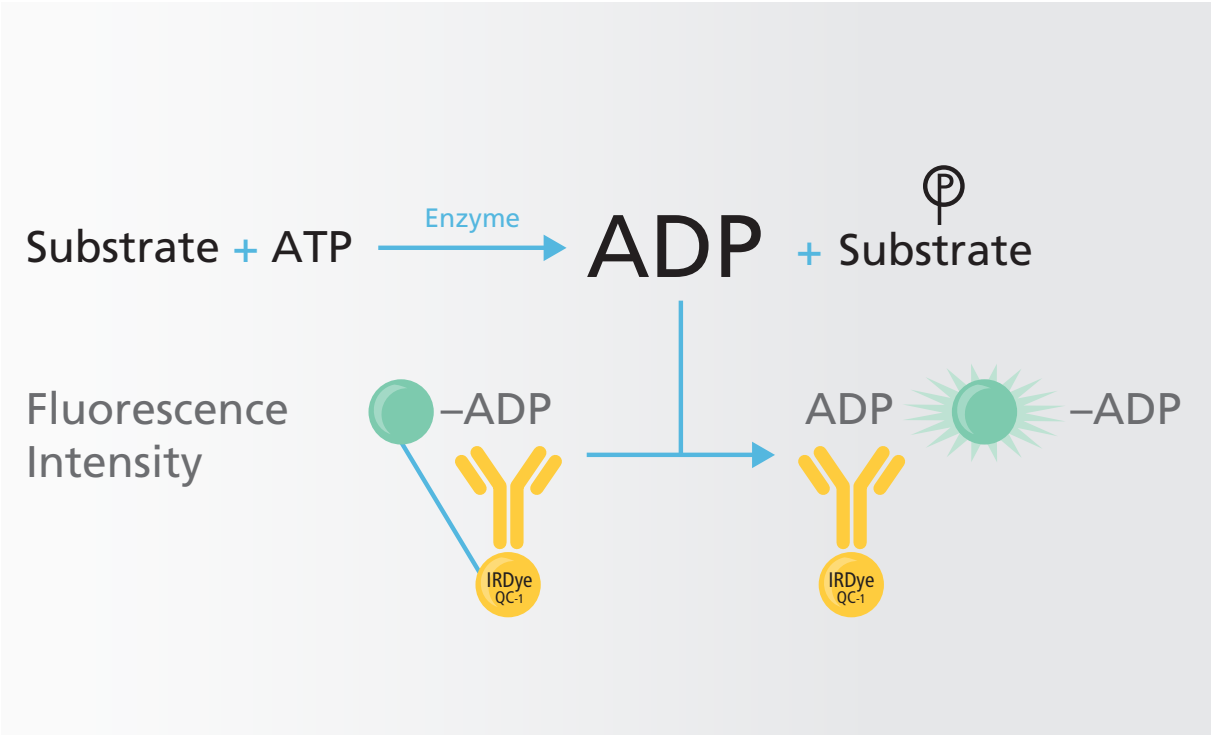


Figure 1: Basic principle underlying the Transcreener® ADP2 Fluorescence Intensity Assay

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® ADP2 Fluorescence Intensity Assay Kit (Catalogue No. 3013)
- Black, small-volume 384-well microplate (Greiner 784900)

INSTRUMENT SETTINGS

- Excitation filter: ID-number 37998-01
- Emission filter: ID-number 39422-01

Assay Window

In order to define the maximum fluorescence window for the Mithras² LB 943 plate reader, we measured the fluorescence intensity signal of the low (tracer + antibody) and high (free tracer, no antibody) controls, as described in the technical manual. The mean signals of 24 different wells for each control were as follows:

Sample	Mean RFU	SD
High control (free tracer)	986	42
Low control (tracer + antibody)	21	6
Ratio	**47**	

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

The maximum fluorescence window should exceed 5 for the assay to produce reliable results. Here we determined an assay window of 47, which is significantly larger. It can, therefore, be concluded that the Transcreener® ADP2 Fluorescence Intensity Assay is compatible with the Mithras² LB 943 plate reader.

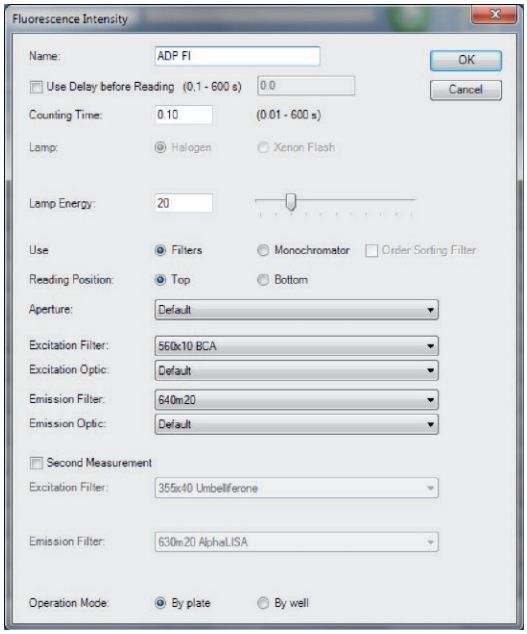


Figure 2: Screenshot of the instrument settings dialogue in the MikroWin 2010 software

Figure 3: Screenshot of the results window in the MikroWin 2010 software. Rows A and B contain the high and low controls, respectively, while rows C to N contain increasing concentrations of ADP.

#	1	2	3	4	5	6	7	8	9	10	11	12	13	14
A	987	951	970	886	988	960	997	960	1062	1062	979	988	942	
B	18	18	18	9	28	28	18	28	28	18	18	18	28	
C	139	129	120	120	129	129	120	129	102	92	102	102	129	
D	397	342	314	342	323	360	369	351	323	369	379	332	332	
E	480	452	480	489	489	526	499	517	508	517	526	517	508	
F	600	582	554	609	619	573	619	609	573	591	591	582	628	
G	685	637	656	609	628	683	674	637	665	656	619	628	693	
H	619	656	656	628	656	600	656	693	720	674	683	683	665	
I	748	674	776	693	720	693	720	702	766	757	748	748	748	
J	813	729	711	766	720	720	748	729	720	729	739	711	748	
K	859	831	831	803	822	803	785	794	813	905	859	840	757	
L	886	813	822	840	859	803	850	886	813	831	868	877	868	
M	850	988	886	859	803	886	896	785	831	877	896	831	942	
N	877	933	923	831	831	942	905	923	942	886	970	886	840	
O	46	37	46	28	28	37	37	46	46	28	46	46	46	
P	37	37	28	46	28	37	37	46	37	46	46	55	18	

ADP/ATP Standard Curve

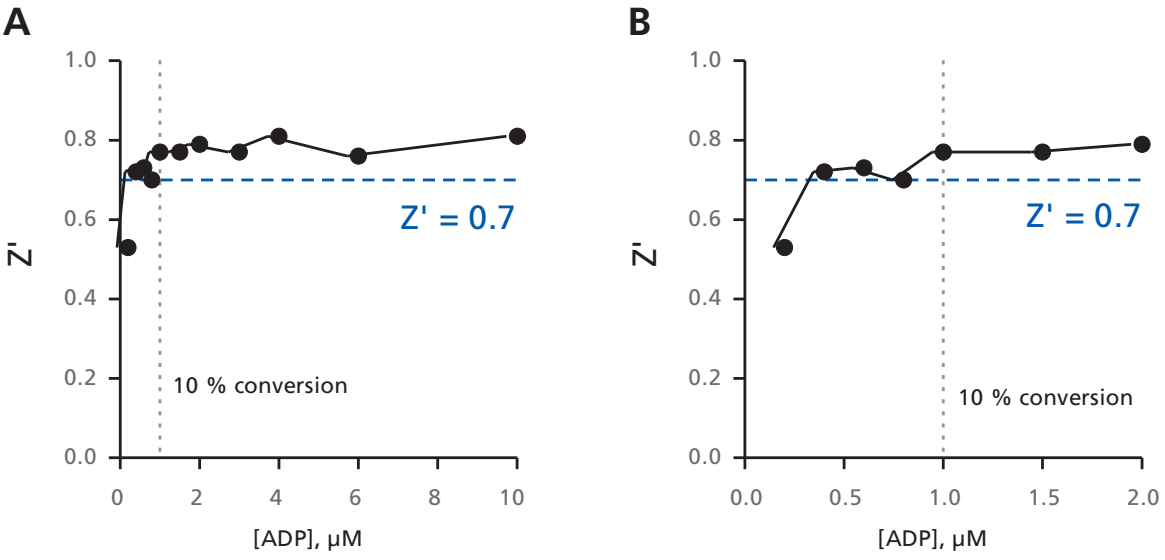
During an enzyme reaction, ATP would be consumed and ADP produced. This reaction was mimicked by assessing different ratios of ADP and ATP, starting from 10 µM ATP / 0 µM ADP to 0 µM ATP / 10 µM ADP. The concentration of adenine thereby remains constant at 10 µM throughout. As the concentration of ADP increases, more of the tracer bound to the antibody will be displaced and released from the influence of the quencher. Thus, a stronger fluorescence signal results. The assay components were as follows:

- ATP/ADP mixture: 4 mM $MgCl_2$, 2 mM EDTA, 50 mM HEPES, pH 7.5, 1% DMSO, 0.01% Brij-35, ATP/ADP (combined to a constant adenine concentration of 10 µM)
- ADP detection mixture: 1X Stop & Detect Buffer B, 8 nM ADP Alexa594 Tracer, 10.0 µg/mL ADP[2] Antibody-IRDye®QC-1
- Positive control: 1X Stop & Detect Buffer B, 8 nM ADP Alexa594 Tracer
- Blank control: 1X Stop & Detect Buffer B, 10 µg/mL ADP2 Antibody-IRDye®QC-1

10 µl of 1X ADP detection mixture was given to 10 µl of ADP/ATP solutions of different ADP/ATP ratios. Thus, a final volume of 20 µl was given to each well of a black, small-volume 384-well plate and incubated for 1 h at room temperature prior to measurement.

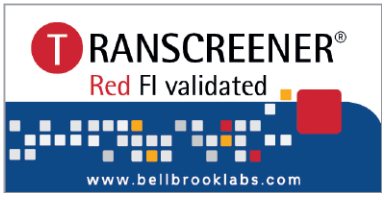
In order to validate an instrument for use with the Transcreener® Fluorescence Intensity Assay, a Z' ≥ 0.7 at 10 % conversion of 10 µM ATP should be obtained. On the Mithras² LB 943 plate reader, the Z' factor at 10% conversion of 10 µM ATP was 0.77 (Figure 4). Thus, the Mithras² LB 943 plate reader is a suitable instrument for use with the Transcreener® ADP2 Fluorescence Intensity Assay.

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



Conclusions

A very large assay window of 47 and a Z' factor of 0.77 at 10 % conversion of 10 µM ATP were obtained, confirming the suitability of the Berthold Technologies Mithras² LB 943 Monochromator Multimode Reader for use with the Transcreener® Fluorescence Intensity Assay.



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detect and identify

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