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detect and identify

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Application Note

VALIDATION OF THE TRISTAR² S LB 942 MULTIDETECTION MICROPLATE 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 TriStar² S LB 942 developed by Berthold Technologies. In order to confirm the compatibility of the Transcreener® Fluorescence Intensity Assay Kit with the TriStar² S LB 942, we have tested the assay controls and an ADP/ATP standard curve that mimics an enzyme reaction. The large assay window of 12 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 TriStar² S LB 942 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.

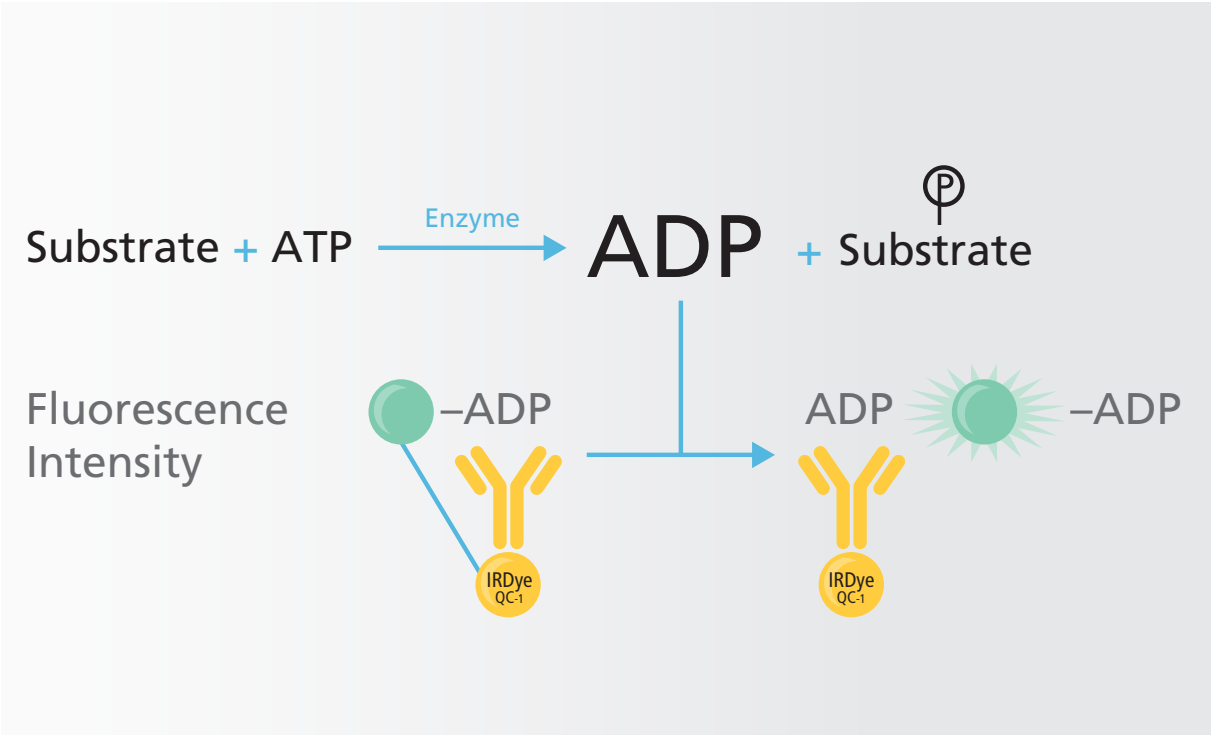


Figure 1: Basic principle underlying the Transcreener® ADP2 Fluorescence Intensity Assay.

THE BELLBROOK LABS TRANSCREENER® FLUORESCENCE INTENSITY ASSAYS

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.

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 S LB 942 Multidetection Microplate Reader
- Transcreener® ADP2 Fluorescence Intensity Assay Kit (Catalogue No. 3013)
- Black, small-volume 384-well microplate (Greiner 784900)

INSTRUMENT SETTINGS

- Excitation filter: ID-number 39804
- Emission filter: ID-number 39422

Assay Window

In order to define the maximum fluorescence window for the TriStar² S LB 942 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)	11462	266
Low control (tracer + antibody)	971	37
Ratio	**12**	

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 12, which is significantly larger. It can, therefore, be concluded that the Transcreener® ADP2 Fluorescence Intensity Assay is compatible with the TriStar² S LB 942 plate reader.

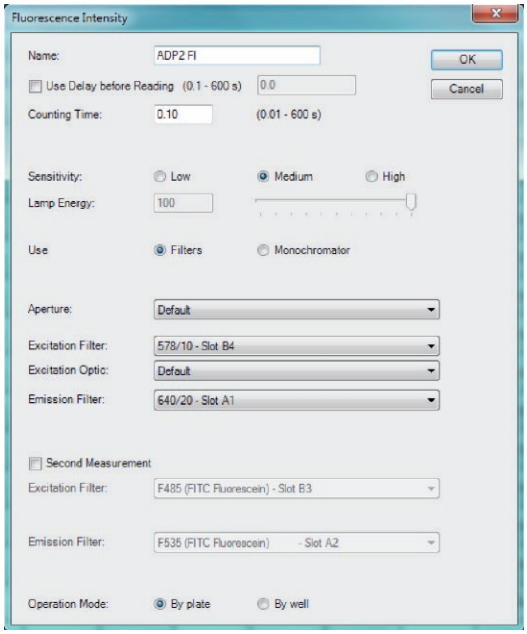


Figure 2: Screenshot of the instrument settings dialogue in the MikroWin 2010 software

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	11908	11242	11053	11162	11436	11853	11305	11361	11533	11333	11569	11389	11710	11593
B	907	995	982	917	992	909	995	1000	1008	1025	940	954	959	907
C	1786	1849	1879	1936	1928	1865	1796	1824	1811	2036	1920	1790	1885	1892
D	4214	4206	4060	4228	4203	4302	4203	4223	3901	4755	3930	4384	4158	4142
E	6145	5925	5855	6183	6042	6460	5453	6053	5568	6606	6691	6327	6134	6196
F	7334	7034	7429	6566	7235	7008	7017	7935	7265	7244	7313	7019	7039	7318
G	8117	8109	8345	8005	7596	8124	8112	7969	7890	7729	7715	8379	7497	7757
H	8556	8317	8520	7740	8513	8384	8110	8738	8253	8763	8266	8582	8287	8284
I	9588	9762	8723	8789	8548	9381	9254	8907	10087	9200	8843	9215	9351	9890
J	9804	9094	8881	8806	8794	8695	8172	9831	8518	9371	9137	9620	9635	9344
K	10194	9988	9991	9775	9563	11307	9226	10246	10165	10038	10183	10047	9911	10218
L	10292	10961	10483	10569	10316	10723	10093	10896	10057	10309	11185	10265	10390	10075
M	10799	10541	10599	10610	10907	10049	10575	10139	10922	11362	10453	10288	10640	10373
N	10804	10597	11256	10329	10396	11438	10636	10710	11724	11083	11011	10980	10487	11414
O	965	956	984	905	991	949	931	1047	889	1007	984	992	942	972
P	995	909	894	971	865	1021	896	975	929	978	1030	896	1006	937

ADP/ATP Standard Curve

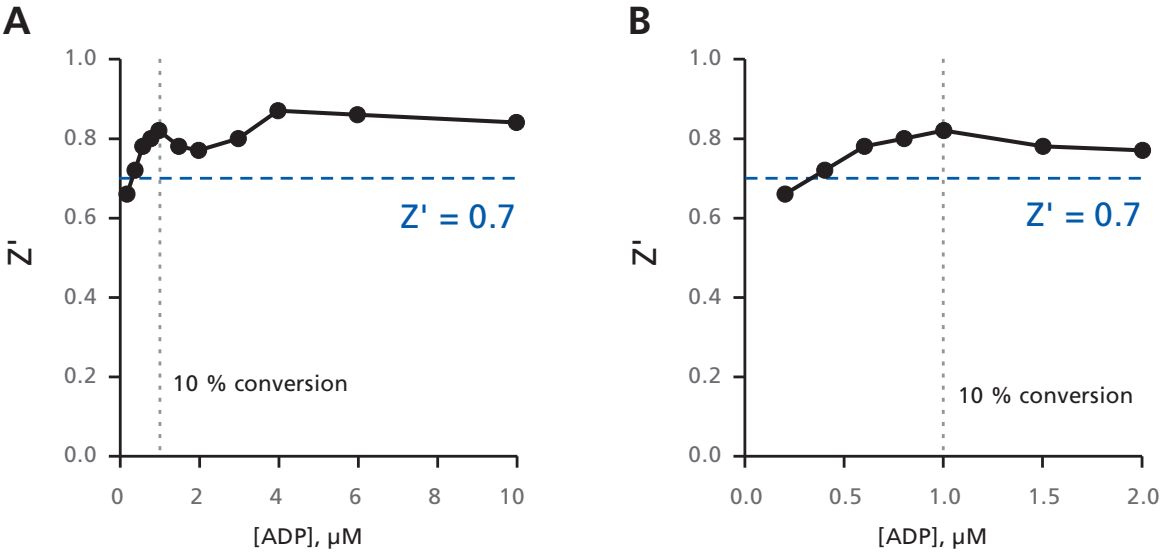
During an enzyme reaction, ATP would be consumed and ADP produced. This reaction was mimicked by assessing different ratios of ADP and ATP, starting from 10 µM ATP / 0 µM ADP to 0 µM ATP / 10 µM ADP. The concentration of adenine thereby remains constant at 10 µM throughout. As the concentration of ADP 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, 19.3 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 TriStar² S LB 942 plate reader, the Z' factor at 10 % conversion of 10 µM ATP was 0.82 (Figure 4). Thus, the TriStar² S LB 942 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 large assay window of 12 and a Z' factor of 0.82 at 10 % conversion of 10 µM ATP were obtained, confirming the suitability of the Berthold Technologies TriStar² S LB 942 Multidetection Microplate Reader for use with the Transcreener® Fluorescence Intensity Assay.