Application Note

VALIDATION OF THE TRISTAR 5 MULTIMODE MICROPLATE READER WITH THE TRANSCREENER® ADP^2 FI ASSAY

Abstract

The quantification of ADP enables to study the regulation of the activity of protein kinases, which influence a wide range of cellular processes. For this application, the Transcreener® ADP2 FI assay offers a very convenient, fast and reliable quantification method. In this study, the performance of the assay in combination with the Tristar 5 Multimode Reader was measured. A very good signal/noise ratio of at least 19 and Z' of 0.75 were obtained. This meets BellBrook Labs' validation criteria and confirms the suitability of this assay-reader combination for quantifying ADP and studying the regulation of protein kinases.

Introduction

Protein phosphorylation and dephosphorylation are among the most common posttranslational modifications that regulate the structures and functions of cellular proteins. They influence a broad spectrum of cellular processes, from the control of cell fate to the regulation of metabolism [1]. In this process, a protein kinase catalyses the transfer of γ-phosphate from ATP (or GTP) to its protein substrates. This phosphorylation then regulates protein functions by inducing conformational changes or interrupting or creating protein-protein interaction surfaces [1]. Aberrant activity of protein kinases is implicated in numerous diseases, ranging from cancer to inflammation and immune disorders [2]. Thus, protein kinases represent important targets for the discovery of new pharmaceutical agents.

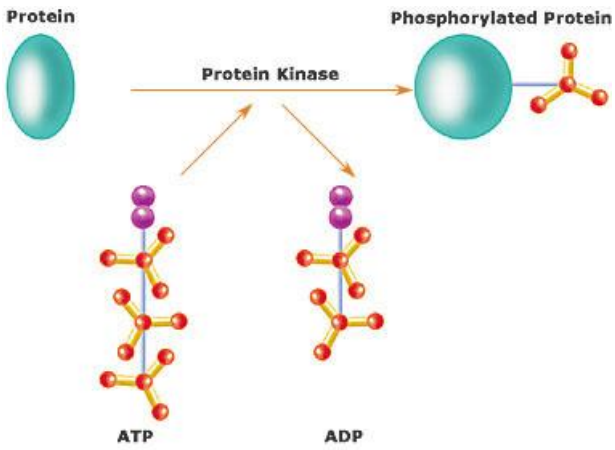

Figure 1. Schematic representation of the activity of protein kinases.

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To study the regulation of protein kinases, it is very important to be able to measure the activity of the kinases of interest. One way to achieve this is to measure the amount of ADP in a solution containing the kinase of interest, its substrate, and ATP: as a consequence of the activity of the protein kinase, phosphate will be transferred from ATP to the substrate and ADP will be released.

BellBrook Labs' Transcreener® assay platform is tailor-made for efficient high-throughput screening (HTS) using a convenient mix-and-read format. It ensures reagent stability and seamless integration with widely used multimode plate readers. The Transcreener® HTS assay platform eliminates the time-consuming process of assay development for novel HTS targets, making compound and inhibitor profiling across various target families significantly more straightforward.

The Transcreener® ADP^2 FI Assay is a red, competitive FI method. Because it is highly selective for ADP, the assay can be used with any enzyme that converts ATP to ADP. It accommodates ATP concentrations ranging from 0.1 µM to 100 µM. The assay delivers excellent data quality (Z' ≥ 0.7) and signal at low substrate conversion. Furthermore, it minimizes interference from fluorescent compounds and light scattering. It is also used by BellBrook Labs as a model assay to validate microplate readers for use with Transcreener® assays.In this application note the performance of the Tristar 5 Multimode Microplate Reader is tested using different measurement settings and compared with the validation criteria of BellBrook Labs.

Tristar 5 Multimode Microplate Reader

Flexibility and sensitivity whenever you need it

The Tristar 5 is a modular high-performance microplate reader equipped with FlexTec Optics, offering you the best of two worlds: independent, user-selectable filters and monochromators on both, the excitation and emission side, for any measurement. This guarantees both, flexibility, and sensitivity whenever you need it.

The Tristar 5 Multimode Microplate Reader provides you with application flexibility for today, tomorrow, and beyond to master your changing plate reading applications:

- High sensitivity luminescence
- BRET
- Absorbance (UV/VIS)
- Fluorescence
- FRET
- TRF, TR-FRET and HTRF®
- AlphaScreen®
- FP

To meet your compliance requirements, a set of validation tools and optional software providing 21 CFR part 11 compliance are available.

Materials

- Tristar 5 “Research Performance FL” Multimode Microplate Reader from Berthold Technologies (Id. Nr. 69185-25).
- Tristar 5 “Advanced Performance” Multimode Microplate Reader from Berthold Technologies (Id. Nr. 69185-15).
- Excitation filter: 578/10 (Id. Nr. 39804). Emission filters: 640/20 (Id. Nr. 39422) and 630/20 (Id. Nr. 50097).
- Transcreener® ADP2 FI Assay Kit from BellBrook Labs (Part Nr. 3013-1K).
- Dilution buffer: 50 mM Tris (pH 7.5), 5 mM $MgCl_2$, 0.01% Brij-35
- Non-Binding black 384-well microplates (small volume) from Greiner (784900).
- Multipette E3 from Eppendorf (Order Nr. 4987000010).
- Pipettes and pipette tips of various sizes.

Readers of the Tristar series can be equipped with two different photomultiplier tubes: the photomultiplier tube installed in standard instruments can measure luminescence and fluorescence up to 650 nm, and models with the “FL” code use a photomultiplier tube that can measure up to 850 nm. Both models are able to measure at the wavelength required by the ADP^2 FI kit (emission maximum of the Alexa 594 tracer is 617 nm). Since both PMTs have a different spectral response, both options were tested.

Instrument settings

- Plate type: Berthold 384 – No:32505
- Measurement Mode: Fluorescence Intensity
- Counting time: 0.2 or 0.1 s
- Sensitivity: 700 V
- Use: Filters
- Aperture: Default
- Excitation: 578/10
- Emission: 630/20 or 640/20
- Operation Mode: By plate

Methods

All reagents were prepared according to the instructions of the manufacturer and brought to room temperature before using them.

To simulate the different ATP-ADP conversion rates which can be found in experiments with protein kinases, a series of 10 µM ATP-ADP mixtures were prepared for the following conversion rates of ATP to ADP: 100% (10 µM ADP), 80%, 60%, 40%, 30%, 20%, 10%, 5%, 2.5%, 1%, 0.5% and 0% (10 µM ATP). 16 replicates of each mixture were measured. To calculate the signal/noise ratio, a mixture without tracer (lowest signal) and one without antibody (maximum signal) were measured in 12 replicates each.

To run the assay, 10 µL of the detection mix were added to all wells. The plate was sealed with a film and shaked for 1 minute.

After incubating the plate for 1 h at room temperature in the dark, the plate was measured using MikroWin 2010 and the settings above.

After measurement, results were exported to Excel format. Signal/noise ratios and Z’ were calculated, and graphs were plotted.

Results

Using the 630/20 emission filter, 0.2 s Counting Time and 700 V sensitivity, both Tristar 5 models achieved a Z' of 0.75 at a 10% ADP conversion, meeting the validation criterium of BellBrook Labs for this kit (Z' at least 0.7 at 10% ADP conversion). Other settings tested either did not improve the results or resulted in poorer assay performance.

The Z' values were quite similar for both Tristar 5 models (see figure 2). However, the signal/noise ratios were different: 20 for the standard model and 35 for the FL one. This is not surprising, because the measurement is performed at a wavelength close to the spectral limit of the standard photomultiplier (650 nm), where quantum efficiency is lower than that of the FL one.

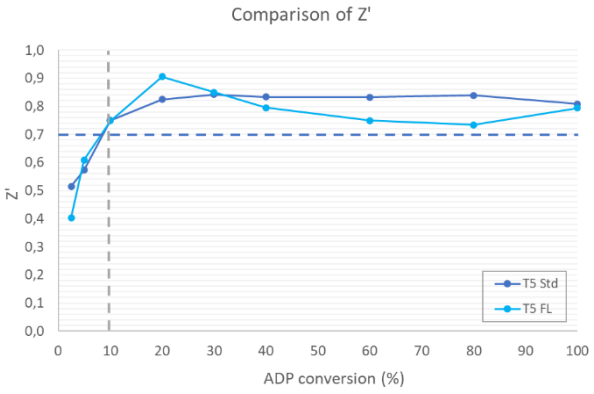

Figure 2. Z' of each Tristar 5 model at different ADP conversion ratios. Dashed lines mark the minimum Z' to pass the validation (0.7, horizontal line) and the reference ADP conversion (10%, vertical line).

Conclusions

Both, the Tristar 5 models equipped with standard PMT as the ones equipped with extended spectral range PMTa are equally suitable and provide good performance.

The combination of the Transcreener® ADP^2 FI Assay with the Tristar 5 multimode reader provides a convenient and reliable way to quantify the conversion of ATP to ADP, as demonstrated by meeting the validation criteria of the manufacturer. This combination is a very valuable tool to study the regulation of protein kinases.

References

1. Cheng HC, Qi RZ, Paudel H, Zhu HJ. Regulation and function of protein kinases and phosphatases. Enzyme Res. 2011;2011:794089. doi: 10.4061/2011/794089. Epub 2011 Dec 13. PMID: 22195276; PMCID: PMC3238372.
2. Srinivasan J, Cload ST, Hamaguchi N, Kurz J, Keene S, Kurz M, Boomer RM, Blanchard J, Epstein D, Wilson C, Diener JL. ADP-specific sensors enable universal assay of protein kinase activity. Chem Biol. 2004 Apr;11(4):499-508. doi: 10.1016/j.chembiol.2004.03.014. PMID: 15123244.

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