Application Note

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

Abstract

The regulation of protein kinase activity, which affects a wide range of cellular processes, can be studied by quantifying ADP. For this application, the Transcreener® ADP^2 FI assay provides 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 Z' of 0.87 was obtained at 10% conversion, meeting 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.

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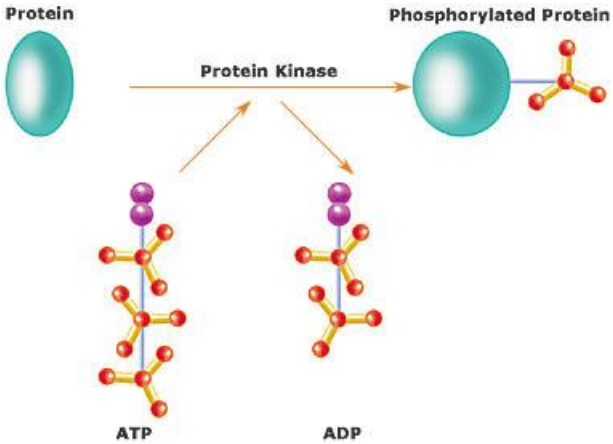

Figure 1. Schematic representation of the activity of protein kinases.

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. To validate an instrument for use with the Transcreener® FP assays, a Z' > 0.7 at 10% conversion of 10 µM ATP was required.

Materials

- Tristar 5 “Research Performance FL” Multimode Microplate Reader from Berthold Technologies (Id. Nr. 69185-25).
- Excitation filter: 578/10 (Id. Nr. 39804). Emission filter: 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
- 384-well Low Volume Black Round Bottom Polystyrene NBS Microplate from Corning (Product Nr. 4514).
- E1-ClipTip™ Electronic Adjustable Tip Spacing Multichannel Equalizer Pipette from ThermoFisher Scientific (Cat. Nr. 4672050BT).
- Pipettes and pipette tips of various sizes.
- D300e Digital Dispenser from Tecan (HP Model Nr. F0L56A).
- Pipettes and pipette tips of various sizes.
- LightCompass® software.

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 ADP² FI kit (emission maximum of the Alexa 594 tracer is 617 nm).

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 hour at room temperature in the dark, the plate was measured using the LightCompass® software and the settings above.

After measurement, results were exported to Excel format. Z’ was calculated and graphs were plotted.

Instrument settings

- Plate type: Costar 384
- Measurement Mode: Fluorescence Intensity
- Counting time: 0.1 s
- Sensitivity: 700 V
- Use: Filters
- Aperture: Default
- Excitation filter: 578/10
- Emission excitation: 630/20
- Operation Mode: By plate

Results

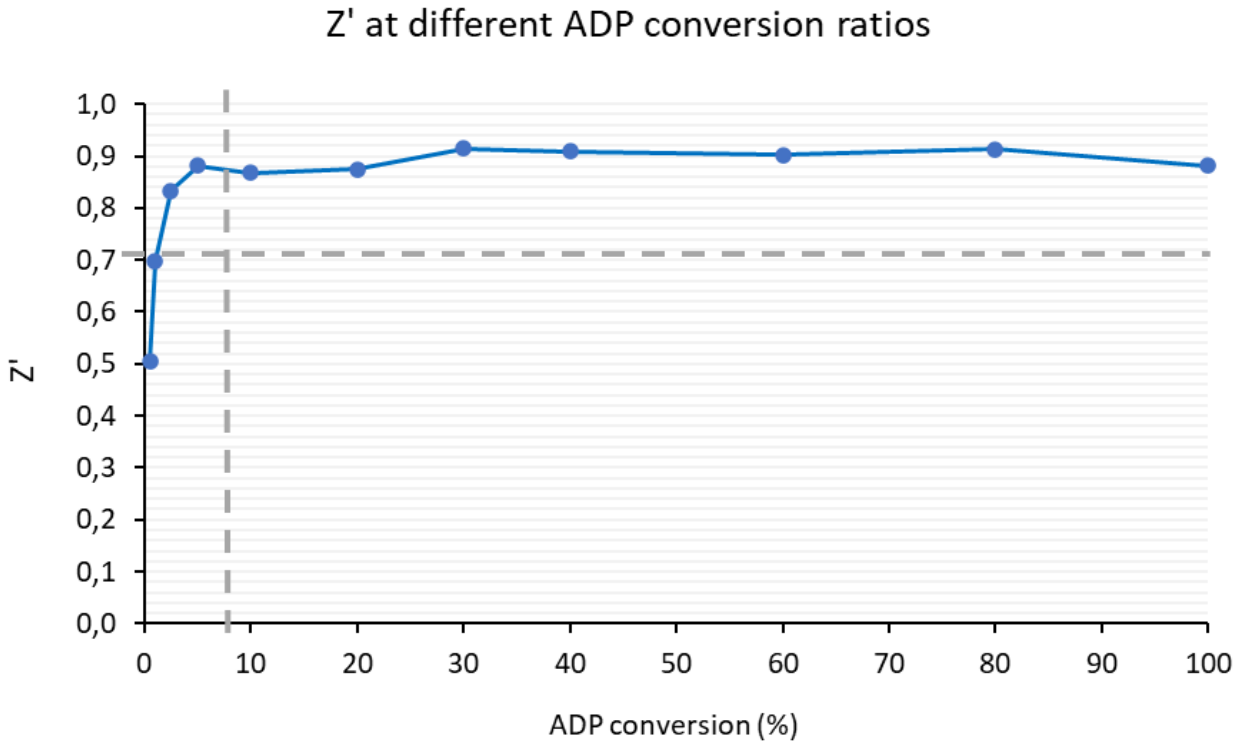

Figure 2. Z' of each Tristar 5 model at different ADP conversion ratios. Dashed lines mark the minimum Z' to pass the validation (0.7, horizontal line) and the reference ADP conversion (10%, vertical line).

Using the 630/20 emission filter, 0.1 s Counting Time and 700 V sensitivity, a Z' of 0.87 at a 10% ADP conversion was achieved, 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 significantly. The Z' values were comparable between standard and "FL" models (data not shown).

Conclusions

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. Both, the Tristar 5 models equipped with standard PMT as the ones equipped with extended spectral range PMT are suitable and provide good performance. This combination is a very valuable tool to study the regulation of protein kinases.

References

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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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