Application Note

OPTIMIZED SETTINGS FOR PROTEIN KINASE ACTIVITY SCREENING USING THE TRISTAR 5 MULTIMODE READER AND THE TRANSCREENER® ADP^2 TR-FRET RED 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. The Transcreener® ADP2 TR-FRET Red Assay provides a very convenient, rapid and reliable method for the assessment of protein kinase activity. In this application note, the performance of the assay was validated in combination with the Tristar 5 Multimode Reader. An assay window of 7.35 and a Z' of 0.89 were determined. 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 post-translational modifications. They regulate the structure and function of cellular proteins. They affect a wide range 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 by disrupting or creating protein-protein interaction surfaces [1]. Dysfunctional protein kinase activity has been implicated in many diseases, ranging from cancer to inflammation and immune disorders [2]. Protein kinases are therefore important targets for the discovery of new drugs.

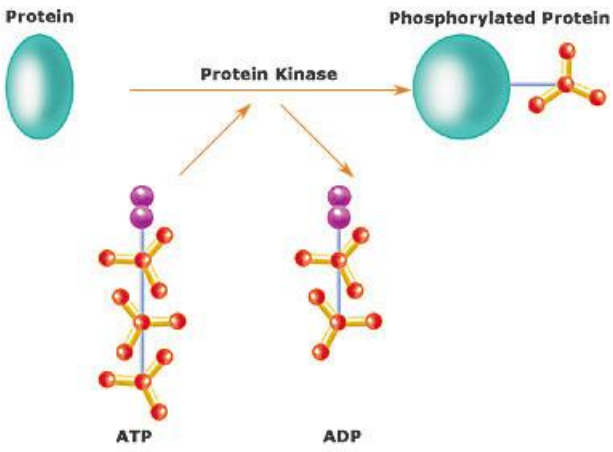

Figure 1. Schematic representation of the activity of protein kinases.

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In order to study the regulation of protein kinases, it is of the utmost importance to be able to measure the activity of the kinases of interest. One method of achieving 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, resulting in the release of ADP.

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. This 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 TR-FRET Red Assay is a competitive immunoassay for ADP with a far-red, TR-FRET readout. The assay is highly selective for ADP and can therefore be used with any enzyme that converts ATP to ADP for a wide range of initial ATP concentrations between 0.1 µM and 1000 µM. The result has excellent data quality (Z' ≥ 0.7) and signal at low substrate conversion. Time-gated detection method and far-red fluorescence largely eliminate interference that can result from prompt fluorescence of test compounds and light scattering. The assay 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).
- HTRF® filter set (Id. Nr. 62772).
- Transcreener® ADP² TR-FRET Red Assay Kit from BellBrook Labs (Part Nr. 3011-1K).
- Dilution buffer: 50 mM Tris (pH 7.5), 5 mM $MgCl_2$, 0.01% Brij-35
- 384-well Low Volume White Round Bottom Polystyrene NBS Microplate from Corning (Product Nr. 4513).
- 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. The emission of the acceptor is measured at 665 nm. Therefore, only the FL models can measure at the wavelength required for the ADP2 TR-FRET Red assay.

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.

The assay was performed by adding 10 µL of the detection mix 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 below.

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

Instrument settings

- Plate type: Costar 384
- Measurement Mode: TRF (Time-Resolved Fluorescence)
- Counting time: 0.1 s
- TRF Cycle Time: 5000 µs
- TRF Delay Time: 50 µs
- TRF Reading Time: 300 µs
- Use: Exc. Filter / Ems. Filter
- Aperture: Default
- Excitation: 340/26 (HTRF Tb cryptate)
- Excitation Optic: 3 – Wider filter 0.45 mm
- Emission filter (acceptor): 665/7uv (HTRF XL665/APC)
- Emission filter (donor): 620/10uv (HTRF Eu cryptate)
- Operation Mode: By plate

Results

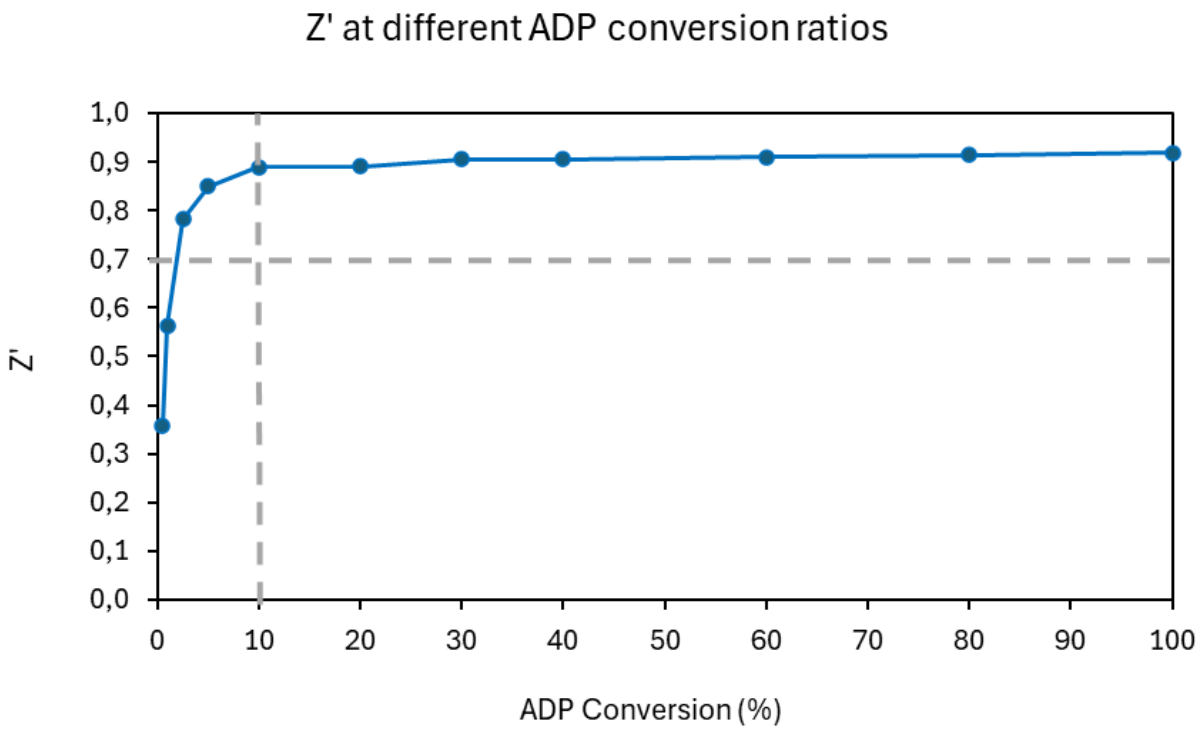

Figure 2. Z' at different ADP conversion ratios. Dashed lines mark the minimum Z' to pass the validation (0.7, horizontal line) and the reference ADP conversion (10%, vertical line). Z' were calculated using 16 replicates for each conversion %.

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Using the settings above, the Tristar 5 achieved an assay window of 7.35 and a Z' of 0.89 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 were tested, but the best performance was obtained with the settings detailed in the "instrument settings" section.

Conclusions

This application note demonstrates the validation of the Berthold Tristar 5 multimode microplate reader for use with the Transcreener® ADP2 TR-FRET Red Assay. The combination of the assay with the Tristar 5 multimode reader provides a convenient and reliable 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.
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