APPLICATION NOTE

VALIDATION OF THE BERTHOLD TECHNOLOGIES MITHRAS² LB 943 MONOCHROMATOR MULTIMODE READER WITH THE ALPHASCREEN® PHOSPHOTYROSINE (P-TYR-100) ASSAY

Abstract

The AlphaScreen® Phosphotyrosine (P-Tyr-100) assay is a bead-based, homogeneous proximity system that can be used for the detection of highly-versatile biomolecular interactions. For the detection of the signal, a suitable plate reader is required, such as the Mithras² LB 943. In order to confirm the compatibility of the AlphaScreen® Phosphotyrosine (P-Tyr-100) assay with the Mithras² LB 943, a biotinylated-LCK-P standard curve that mimics the outcome of a biological reaction was tested. A very large signal-to-background ratio of 300 and a robust standard curve with an EC50 value of 2.97 nM were obtained. A Z'-factor of 0.82 was calculated and the limit of detection determined to be <50 amol/well. These results confirm that the Mithras² LB 943 is a suitable device for measuring the AlphaScreen® Phosphotyrosine (P-Tyr-100) assay.

Introduction

AlphaScreen® is a non-radioactive, bead-based, homogeneous proximity assay to study biomolecular interactions. The acronym "Alpha" stands for Amplified Luminescent Proximity Homogeneous Assay. Binding of molecules captured on the beads leads to an energy transfer from one bead to the other, ultimately producing a luminescent/fluorescent signal. The assay involves two different bead types: donor beads and acceptor beads. Donor beads contain the photosensitizer phthalocyanine. When exposed to light at a wavelength of 680 nm, it converts ambient oxygen to singlet oxygen, an excited and reactive form of O_2. Within its 4 µsec half-life, singlet oxygen can diffuse approximately 200 nm in solution. If an acceptor bead is within that proximity, energy is transferred from the singlet oxygen to thioxene derivatives within the acceptor bead, which produce light at wavelengths between 520-620 nm. In the absence of an acceptor bead in close proximity, singlet oxygen falls to its ground state and no signal is produced. This kit contains streptavidin Donor beads and AlphaScreen Acceptor beads conjugated with the antiphosphotyrosine P-Tyr-100 antibody, and can be used to capture biotinylated phosphoproteins, biotinylated phosphorylated peptides, or biotinylated antibodies in complex with phosphorylated proteins.

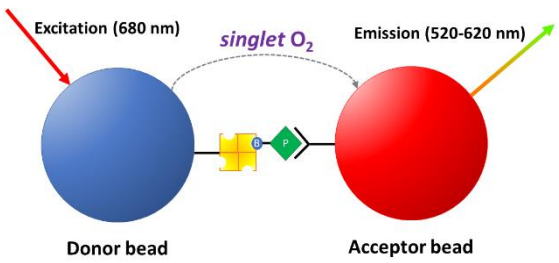

Figure 1: Basic principle underlying the AlphaScreen® Phosphotyrosine (P-Tyr-100) assay. B: Biotin; P: Phosphorilated protein or peptide.

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The Berthold Technologies Mithras² LB 943 Monochromator 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

Materials

- Berthold Technologies Mithras² LB 943 Monochromator Multimode Reader.
- PerkinElmer AlphaScreen® Phosphotyrosine (P-Tyr-100) assay kit (catalogue no. 6760620C).
- White, opaque, small-volume 384-well microplate (Corning 4513).

Instrument settings

- Excitation filter: none (laser used for excitation).
- Emission filter: Alphascreen® filter 565 nm (reference: 40991-01).

A screenshot of instrument settings is shown in Figure 2.

Methods

The 10x buffer supplied with the kit was diluted by adding 500 µl to 4.5 ml Milli-Q® water. Subsequently, 5 mg bovine serum albumin (BSA) was added and the pH adjusted to 7.4. A dilution series at half-log intervals of the provided biotin-LCK-P in 1x buffer was prepared, with the concentrations ranging from 1 µM to 50 pM. 5 µl of each dilution (or buffer only as control) was given to a white, opaque, small-volume 384-well microplate (Corning 4513), followed by 10 µl of the prepared 1x buffer. Under reduced light conditions, 5 µl of the donor and acceptor beads were each given to 245 µl of 1x buffer. Subsequently, 200 µl of the donor bead solution was mixed with 200 µl of the acceptor bead solution. 10 µl of the mixed bead solution was pipetted into each well. The final volume in each well was thus 25 µl. The microplate was incubated in the dark at 25 °C for 120 minutes and analysed using the Mithras² LB 943 Monochromator Multimode Reader.

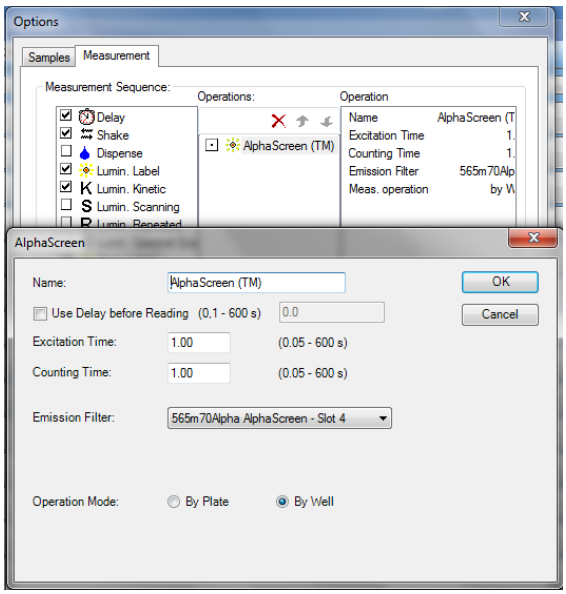

Figure 2: Screenshot of the instrument settings dialogue in the MikroWin 2010 software

Results

A standard curve of different concentrations of the biotinylated probe biotin-LCK-P supplied with the assay kit was assessed to confirm the suitability of the Mithras² LB 943 Monochromator Multimode Reader for the detection of the AlphaScreen® signal. The standard curve is shown in Figure 3.

The mean maximum and minimum assay signals are 2,586,244 cps and 8,633 cps, respectively. This yields a very large assay window of 300 and a Z'-factor of 0.82. The biotin-LCK-P standard curve is robust and has an EC50 value of 2.97 nM, which is similar to the EC50 stated by the kit manufacturer. The limit of detection was determined to be <50 amol/well. These results validate the performance of the AlphaScreen® assay on the Mithras² LB 943 Monochromator Multimode Reader.

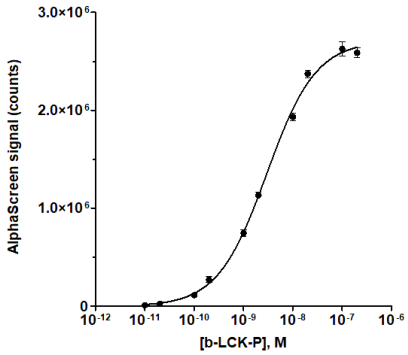

Figure 3. Standard curve of biotin-LCK-P measured on the Mithras² LB 943 Monochromator Multimode Reader. The EC50 value is 2.97 nM.

Conclusions

A 300-fold signal-to-background ratio with a Z'-factor of 0.82, a detection limit of <50 amol/well, and a robust biotin-LCK-P standard curve were obtained, confirming the suitability of the Berthold Technologies Mithras² LB 943 Monochromator Multimode Reader for use with the AlphaScreen® Phosphotyrosine (P-Tyr-100) assay.

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