

AlphaScreen® Phosphotyrosine Assay Performed on a FLUOstar Omega and a POLARstar Omega

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Application Note 186

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- AlphaScreen® tyrosine kinase assay performed on a FLUOstar Omega and POLARstar Omega
- Z' values of > 0.8 indicate a highly robust assay combined with high quality instrumentation
- Sensitivity determined to be ≤ 100 amol biot-LCK-P per well

Introduction

Tyrosine kinases are important regulators of cellular processes that include cell cycle progression, metabolism, and apoptosis. Kinases have been found to be involved in e.g. cancer and cardiovascular diseases; therefore, molecules that modulate kinase functions are expected to be promising new drugs.

There are different homogeneous technologies which can be used to perform kinase assays. In this application note we will describe the performance of a tyrosine kinase assay using the AlphaScreen® (amplified luminescent proximity homogeneous assay) method performed on a FLUOstar Omega and POLARstar Omega.

Assay Principle

The AlphaScreen® assay uses the diffusion of singlet state oxygen from Donor to Acceptor beads. Upon excitation at 680 nm of Donor beads ambient oxygen is converted into singlet oxygen released at a rate of up to 60,000 molecules per second. Singlet oxygen molecules have a short lifetime (4 μ s in aqueous solutions) and diffuse of no more than 200 nm. When a biomolecular interaction brings the Donor and Acceptor beads in proximity, the singlet oxygen reaches the Acceptor bead and a cascade of chemical reactions is initiated producing a greatly amplified luminescence signal in the range of 520 - 620 nm. The AlphaScreen® P-Tyr-100 assay (figure 1) is based on a sandwich assay principle.

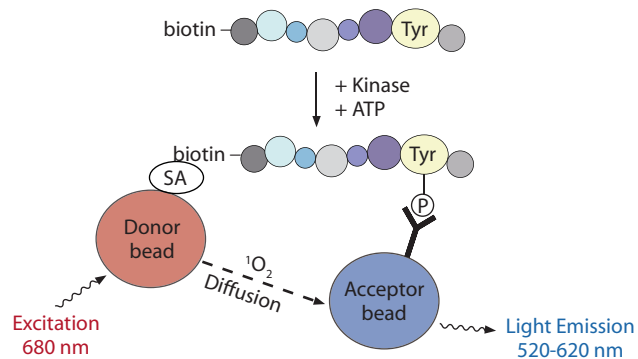


Fig. 1: Principle for an AlphaScreen® tyrosine kinase assay

After tyrosine kinase phosphorylation, a biotinylated polypeptide substrate is sandwiched between a streptavidin(SA)-coated Donor bead and an anti-phosphotyrosine antibody conjugated Acceptor bead. Phosphorylation of the polypeptide by the tyrosine kinase results in an increase in the luminescence signal.

Materials and Methods

- FLUOstar / POLARstar Omega, BMG LABTECH, Offenburg, Germany
- P-Tyr-100 assay kit, PerkinElmer, USA, #6760620C
- White 384-well small volume plates, Greiner Bio-One, Germany, #784075

The P-Tyr-100 (Phosphotyrosine) assay kit was performed in AlphaScreen® mode in accordance with the kit protocol¹ in white 384-well small volume plates with a final assay volume of 17 μ L. Donor and Acceptor beads were used at a final concentration of 20 μ g/mL. The AlphaScreen® components are light sensitive; therefore, the beads should not be exposed to bright light. Beads are best handled under subdued or green filtered light. Plates were sealed and read after one hour incubation at room temperature. The FLUOstar/POLARstar Omega instrument settings for a 384-well plate can be found below.

Instrument Settings

Measurement method	AlphaScreen®
Reading mode	Endpoint
Filters	Excitation: Ex AS, Emission: Em AS
Optic	2 mm top optic

General Settings

Positioning delay	0.50 s
Excitation time	0.30 s
Integration start	0.34 s
Integration time	0.60 s

Results and Discussion

A nine point titration curve with biotinylated and phosphorylated polypeptide (biot-LCK-P) covering concentrations from 50 nM to 5 pM was performed to demonstrate the kit performance (Figure 2).

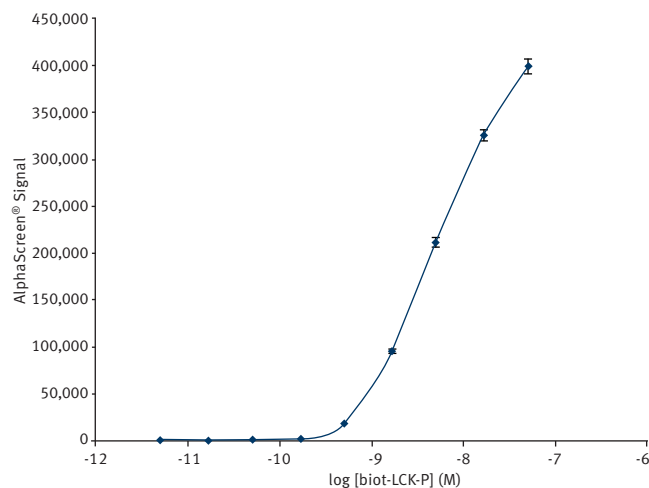


Fig. 2: A typical biot-LCK-P titration curve recorded on a POLARstar Omega in AlphaScreen® mode

The resulting titration curve (Figure 2) very closely corresponds to the curve published in the kit protocol.¹ In order to show that there is no significant well to well variation, the assay was performed with 24 replicates for each concentration and blank. Figure 3 shows the high consistency of well to well measurements.

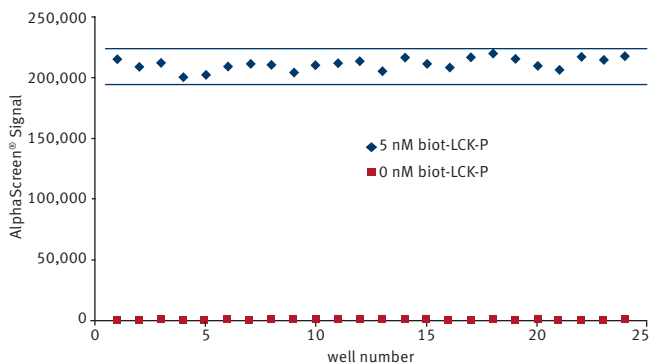


Fig. 3: AlphaScreen® values for 24 replicates at a constant concentration (5 nM) of biotinylated and phosphorylated LCK and a control

The %CV for concentrations between 0.5 and 50 nM were calculated to be < 5%. The Z' value in this concentration range was > 0.8 indicating excellent assay performance.² The limit of detection was calculated to be ≤ 100 amol biot-LCK-P per well.

Conclusion

The AlphaScreen® tyrosine kinase assay was successfully performed on a FLUOstar Omega and a POLARstar Omega in 384-well format. Although there is no laser available, special filters that are optimized for AlphaScreen® provide the possibility to obtain sensitive and consistent data.

The POLARstar and FLUOstar Omega are versatile, automated microplate readers that offer a range of detection modes: UV/Vis absorbance spectra, fluorescence intensity, fluorescence polarization (only POLARstar Omega), time-resolved fluorescence, time-resolved FRET, luminescence (flash and glow) and AlphaScreen®. Top and bottom plate reading, well scanning, precise temperature control and multi-mode shaking capabilities enhance the flexibility of the readers (Figure 4).



Fig. 4: FLUOstar Omega and POLARstar Omega multidetection microplate readers

The Omega instruments are also certified for the Dual Luciferase Reporter (DLR™) gene assay, for the HTRF® assay platform, and has the LanthaScreen™ certified plus status.

References

- 1 AlphaScreen® Phosphotyrosine (P-Tyr-100) Assay Kit Protocol #6760620, PerkinElmer, USA.
- 2 Zhang, J. et al.: (1999) J. Biomol. Screen. **4**, 67-73.

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