Detection of tyrosine kinase activity in AlphaScreen® mode

Franka Ganske and Marjan Orban
BMG LABTECH

- **AlphaScreen® tyrosine kinase assay performed using either a laser or a flash lamp-equipped microplate reader**
- **high Z' values 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.

Assay Principle

The AlphaScreen® assay uses the diffusion of singlet state oxygen from Donor to Acceptor beads. Upon laser 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 µs 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.

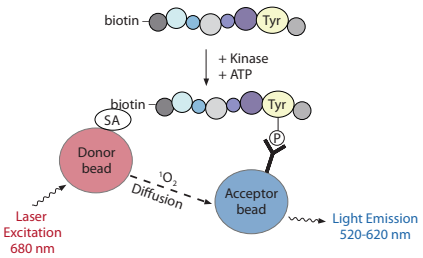

Fig. 1: Principle for an AlphaSreen® tyrosine kinase assay.

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 & Methods

- P-Tyr-100 assay kit, PerkinElmer
- White 384-well small volume plates, Greiner Bio-One

The P-Tyr-100 (Phosphotyrosine) assay kit was performed in AlphaScreen® mode in accordance with the kit protocol in white 384-well small volume plates with a final assay volume of 17 µL. Donor and Acceptor beads were used at a final concentration of 20 µg/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 read after an hour incubation at room temperature. The instrument settings for a 384-well plate can be found below.

Instrument settings

	FLUOstar®/ POLARstar® Omega	CLARIOstar®	PHERAstar® *FS*
Detection mode	AlphaScreen®		
Method	Endpoint, Top optic		
Optic settings	Ex-Filter: EX AS Em-Filter: Em AS	Ex-Filter: EX AS Em-Filter: Em AS	AlphaScreen Optic module
Excitation time	0.3 seconds		
Integration start	0.34 seconds		
Integration time	0.3 seconds		

Results & 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 and Figure 3).

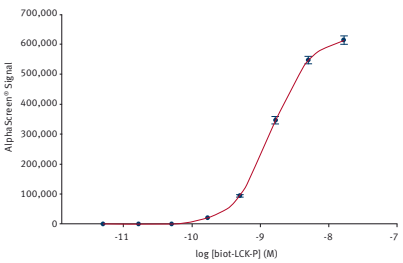

Fig. 2: A typical biot-LCK-P titration curve recorded on the PHERAstar *FS* in AlphaScreen® mode.

Rev. 12/2014
Keywords: **Acceptor beads, AlphaScreen, Beads, Signaling, Tyrosine kinase**

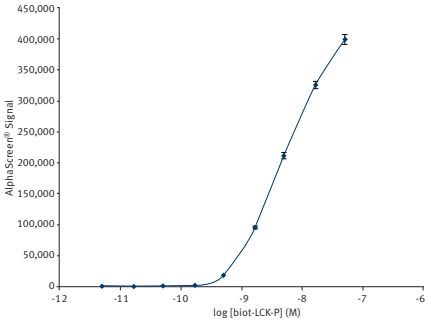

Fig. 3: A typical biot-LCK-P titration curved recorded on the POLARstar Omega in AlphaScreen mode.

The resulting titration curves in Figure 2 and 3 very closely correspond to the curve published in the kit protocol. 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 4 shows the high consistency of well to well measurements.

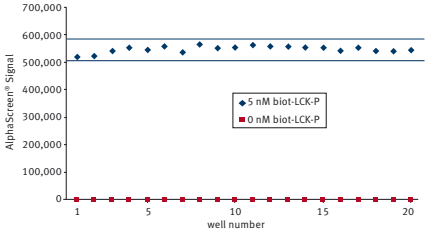

Fig. 4: AlphaScreen® values for 20 replicates at a constant concentration (5 nM) of biotinylated and phosphorylated LCK and a control containing no protein. Data was measured on a PHERAstar *FS*.

Figure 4 shows the high consistency of well to well measurements. The resulting 2.2 %CV (for 5 nM biot-LKC-P) also demonstrates stable measurements. From these assay data, a representative Z' value of 0.93 and an LOD (limit of detection) of ≤100 amol biot-LCK-P per well were calculated.

Conclusion

AlphaScreen® tyrosine kinase assays performed on the PHERAstar *FS* result in very consistent values for replicate wells. As a characteristic parameter for the quality of the assay, a Z' value of 0.93 was calculated, which represents an excellent assay performance. Z' values between 0.5 and 1 indicate a highly robust screening assay and reflect high quality of the instrumentation.
The assay was as well 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 Z´-value for the Omega readers were calculated to be > 0.8 while the limit of detection was calculated to be ≤100 amol biot-LCK-P per well.

For the PHERAstar *FS*, the CLARIOstar and the Omega series of readers the limit of detection was identical. This leads to the strong assumption that the assay itself is limiting.

PHERAstar® *FSX*

*The PHERAstar *FSX* is the newest PHERAstar reader.

CLARIOstar®

Omega Series