# Detection of tyrosine kinase activity using the PHERAstar in AlphaScreen™ mode

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## 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 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 and an AlphaScreen™ specific excitation laser on BMG LABTECH's PHERAstar.
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 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.

### Assay Principle

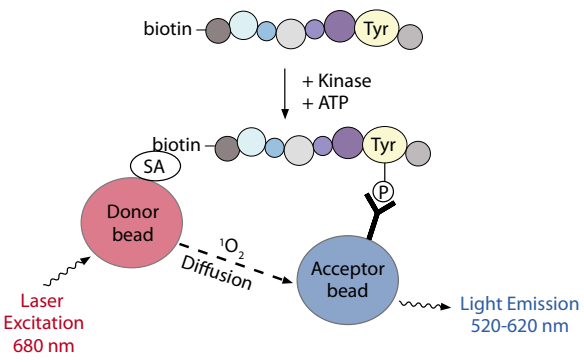


**Fig. 1:** Principle for an AlphaScreen™ tyrosine kinase assay.

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 & Methods

- PHERAstar, BMG LABTECH GmbH, Germany
- P-Tyr-100 assay kit, PerkinElmer, USA, #6760620
- White 384-well small volume plates, Greiner Bio-One, Germany, #784075

The P-Tyr-100 (Phosphotyrosine) assay kit was performed in AlphaScreen™ mode on the PHERAstar 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 with an integration time of 0.3 seconds per well. The instrument settings for a 384-well plate can be found below.

**Instrument Settings**

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|---|---|
| Measurement method | AlphaScreen™ |
| Reading mode | Endpoint |
| Optic module | AlphaScreen™ 680 570 |

**General settings**

| | |
|---|---|
| Positioning delay | 0.10 s |
| Excitation time | 0.30 s |
| Integration start | 0.34 s |
| Integration time | 0.30 s |
| Gain | 3600 |

The PHERAstar's focal height adjustment feature guarantees optimal sensitivity.


**Fig. 1:** BMG LABTECH's PHERAstar multimode microplate reader

## Results & Discussion

To demonstrate the functionality of the AlphaScreen™ assay and the performance on the PHERAstar, a titration curve with a biotinylated and phosphorylated polypeptide (biot-LCK-P) was performed with the anti-phosphotyrosine antibody (Figure 2).

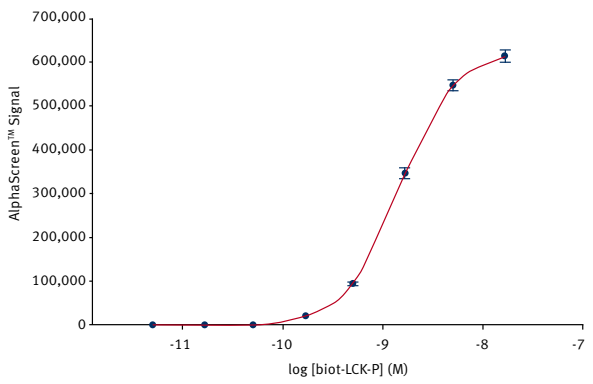


**Fig. 2:** A typical biot-LCK-P titration curve recorded on the PHERAstar in AlphaScreen™ mode

The concentration of biot-LCK-P used was in the range of 5 pM to 17 nM and the final assay volume was 17 µL per well. 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 same assay was performed with 20 replicates at both a single biot-LCK-P concentration (5 nM) and a control concentration (without biot-LCK-P).

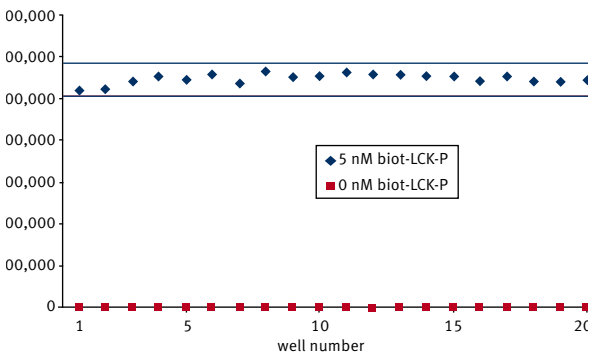


**Fig. 3:** AlphaScreen™ values for 20 replicates at a constant concentration (5 nM) of biotinylated and phosphorylated LCK and a control containing no protein

Figure 3 shows the high consistency of well to well measurements when using the PHERAstar. The resulting 2.2 %CV ( for 5 nM biot-LKC-P) also demonstrates consistent 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 result in very consistent values for replicate wells. The limit of detection was determined to be ≤ 100 amol biot-LCK-P per well. 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.[2]
In drug discovery, successful detection strategies have to be compatible with minituarized HTS. The multimode HTS reader PHERAstar shows great performance in AlphaScreen™ mode as demonstrated with the tyrosine kinase assay in 384-well small volume plate format. The easy to use software allows simple assay optimization regarding sensitivity and read times. Assay flexibility is further enhanced by precise temperature control to 45°C as well as multimode shaking capabilites. Both are standard on the PHERAstar.
The PHERAstar has been designed to read all HTS detection modes (fluorescence intensity, time-resolved fluorescence, fluorescence polarization, luminescence, AlphaScreen™, and absorbance) in all plate formats up to 1536 wells.

## 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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