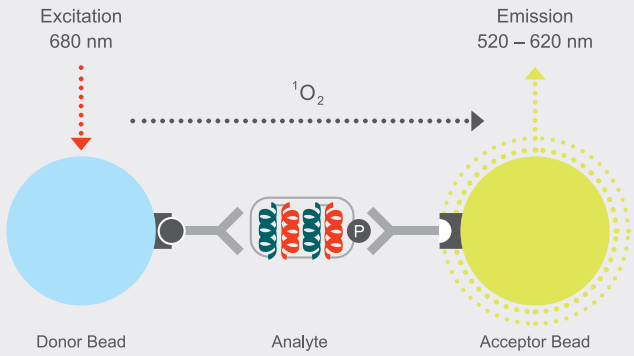

Implementation of AlphaScreen® technology on the Infinite® F200 PRO

Detection of tyrosine kinase activity using the Infinite F200 PRO's new AlphaScreen function

Introduction

AlphaScreen (Amplified Luminescent Proximity Homogeneous Assay) is a bead-based screening technology developed for fast, reliable and cost-effective detection of biomolecular interactions. It utilizes the energy transfer between donor and acceptor beads that occurs when these are brought into close proximity due to a binding event between their coupling partners. As a result, a strong luminescent signal is generated that can be detected in a wavelength range of 520-620 nm [1].

Tyrosine kinases are important mediators of cellular processes such as signal transduction, cell growth and apoptosis. They have been reported to be involved in a number of diseases associated with excessive cell proliferation – including atherosclerosis and cancer – and are therefore often targeted in drug development and high-throughput screening (HTS) approaches.

AlphaScreen-based phosphotyrosine assay kits, for example P-Tyr-100, have been developed for reliable and sensitive detection of kinase activity. The AlphaScreen signal is dependent on the extent of tyrosine kinase phosphorylation.[2].

The Infinite F200 PRO is one of Tecan's most reliable and sensitive filter-based multimode readers. It features all common measurement modes, including absorbance, fluorescence top/bottom, single and dual luminescence, fluorescence polarization (FP) and time-resolved fluorescence resonance energy transfer (TR-FRET) techniques such as HTRF®. In addition, the Infinite F200 PRO is now capable of measuring AlphaScreen- and AlphaLISA®-based assays.

This Application Note describes the implementation of the AlphaScreen assay technology on the Infinite F200 PRO and its use for the detection of tyrosine kinase activity using the AlphaScreen Phosphotyrosine (P-Tyr-100) Assay Kit [2].

Materials and methods

- P-Tyr-100 Assay Kit (PerkinElmer, #6760620)
- 384-well small volume microplates (Greiner®, #784075)

Pipetting of the AlphaScreen reagents was performed under light-protected conditions (<50 lux) to prevent the components from photobleaching, and the P-Tyr-100 Assay Kit was used according to the manufacturer's instructions. Briefly, a dilution series of biotinylated and phosphorylated LCK peptide (bio-LCK-P) was prepared, and replicates of each concentration were pipetted into a 384-well small volume microplate (20 µl/well); blanks consisting of assay buffer without bio-LCK-P were also included. AlphaScreen donor and acceptor beads were diluted to a working concentration of 20 µg/ml, pre-mixed as described in the assay protocol, and added to the samples and blank wells.

In addition, replicates of one selected bio-LCK-P concentration were distributed across a separate microplate to assess the AlphaScreen measurement uniformity. The plates were incubated at room temperature for 1 h in the dark, and then measured on the Infinite F200 PRO in AlphaScreen mode using the settings summarized in Table 1.

Measurement parameters	
Plate definition file (PDFX)	GRE384sw
Excitation filter (bandwidth)	680 (30) nm
Emission filter (bandwidth)	570 (100) nm
Excitation time	1000 ms
Integration time	500 ms
Gain	Calculated from a well with the highest signal, or pre-optimized and set manually
Settle time	0 ms

Table 1 AlphaScreen measurement settings on the Infinite F200 PRO

The gain was optimized in a quick pre-measurement so that the well containing the highest analyte concentration yielded approximately 55000 RFU, and the determined value was then set manually for measurement of the whole plate. Alternatively, the gain may be optimized based on any well containing the highest assay concentration using the automatic 'calculated from well' gain option.

Results

Figure 1 shows the AlphaScreen signals obtained with the bio-LCK-P dilution series in the Infinite F200 PRO. The measured signal curve has a similar shape to the standard curve published in the P-Tyr-100 kit instructions, exhibiting very small standard deviations even in the low-concentration range and typically resulting in a detection limit of ≤ 100 amol/well bio-LCK-P. The calculated Z' value of 0.90 emphasizes the excellent assay quality [3].

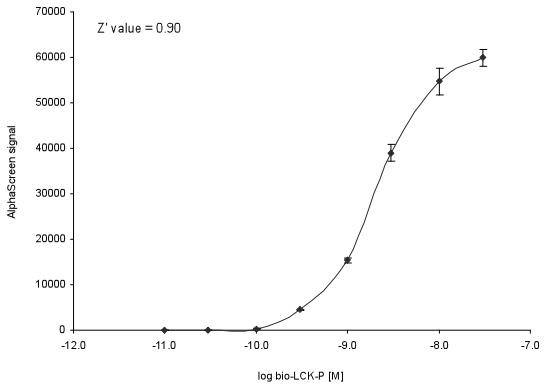

Figure 1 Typical AlphaScreen signal curve of a bio-LCK-P dilution series

To assess the AlphaScreen uniformity, additional microplates containing replicates of 3 nM bio-LCK-P or blanks (assay buffer without bio-LCK-P) distributed across the plate were measured. The results showed only minimal well-to-well variation, with a CV of 2.96 % in the signal wells and 5.68 % in the blank wells, indicating very good signal consistency (Figure 2).

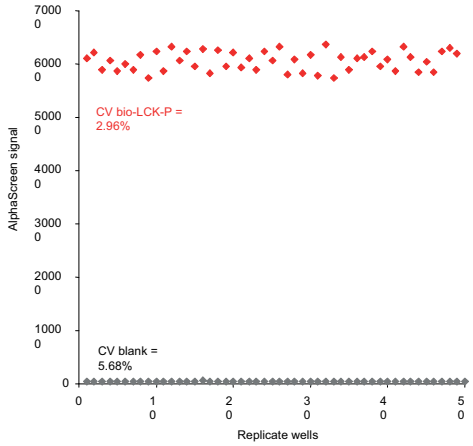

Figure Figure 2 AlphaScreen measurement uniformity using 3 nM bio-LCK-P and blanks

Conclusion

The results summarized in this Application Note demonstrate the excellent performance of the Infinite F200 PRO for AlphaScreen assays such as the P-Tyr-100 assay. The instrument achieves a detection limit of <100 amol/well, with minimal measurement variation in signal and blank wells and an extraordinarily high Z' value of 0.90.

The Infinite F200 PRO's fluorescence optics module, consisting of dedicated AlphaScreen and AlphaLISA filters sets and an optimized dichroic mirror, enables sensitive and reproducible AlphaScreen measurements.

In addition to its well-established multimode reading capacities, the new AlphaScreen/AlphaLISA functionality makes the Infinite F200 PRO perfectly suited to research, assay development and screening.

Abbreviations

Alpha	Amplified Luminescent Proximity Homogeneous Assay
bio-	Biotinylated
CV	Coefficient of variation
FRET	Fluorescence Resonance Energy Transfer
FP	Fluorescence Polarization
HTRF	Homogeneous Time-Resolved Fluorescence
HTS	High throughput screening

References

1) A Practical Guide to Working with AlphaScreen (http://www.urmc.rochester.edu/hts/_source/AlphaScreenPracticalGuide.pdf)
2) Using the AlphaScreen Omnibeads. (http://www.perkinelmer.com/CMSResources/Images/44-73451MAN_AlphaScreenOmniBeads.pdf)
3) Zhang et al. A Simple Statistical Parameter for Use in Evaluation and Validation of High Throughput Screening Assays. J Biomol Screen. 1999;4(2): 67-73 q

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