

New Infinite[®] M1000 PRO with AlphaScreen[®] module

AlphaScreen - based detection of tyrosine kinase activity using the Infinite M1000 PRO

Introduction

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 (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].

The AlphaScreen P-Tyr-100 assay has been developed for reliable and sensitive detection of kinase activity. The AlphaScreen signal is dependent on the extent of tyrosine kinase phosphorylation, increasing with the degree of phosphorylation [2].

The Infinite M1000 PRO is the latest addition to Tecan's high-end microplate reader portfolio. It offers a wide range of reading modes including absorbance, fluorescence top/bottom, single/dual luminescence (including luminescence scanning), fluorescence polarization (FP) and time-resolved fluorescence resonance energy transfer (TR-FRET) techniques such as HTRF[®] and LanthaScreen[™], and now features a high end AlphaScreen module. The new module combines a powerful laser light source and dedicated emission filters to achieve uncompromised performance for AlphaScreen and AlphaLISA[®] assays.

The Infinite M1000 PRO's dedicated luminescence detector is used for ultra-sensitive detection of the Alpha signal. In addition to the advanced optics module, the Infinite M1000 PRO offers an ingenious real-time temperature correction function that compensates for sample temperature variations across the microplate.

This Application Note describes the implementation of the AlphaScreen assay technology on the Infinite M1000 PRO and its use for the detection of tyrosine kinase activity using the AlphaScreen 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 concentration of bio-LCK-P were distributed across a separate microplate to test the AlphaScreen measurement uniformity. The plates were incubated at room temperature for 3 hours in the dark, and then measured on the Infinite M1000 PRO in AlphaScreen mode using the settings summarized in Table 1.

Measurement parameters	
Plate definition file	GRE384sw.pdf (white 384-well small volume plate, Greiner)
Excitation time	100 ms
Integration time	300 ms
Settle time	0 ms
Filter	AlphaScreen
Temperature correction	activated

Table 1 AlphaScreen measurement settings

Results

Figure 1 shows the AlphaScreen signals obtained with the bio-LCK-P dilution series in the Infinite M1000 PRO. The measured signal curve has a similar shape and progress to the standard curve published in the 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 0.97 indicates excellent assay quality [3].

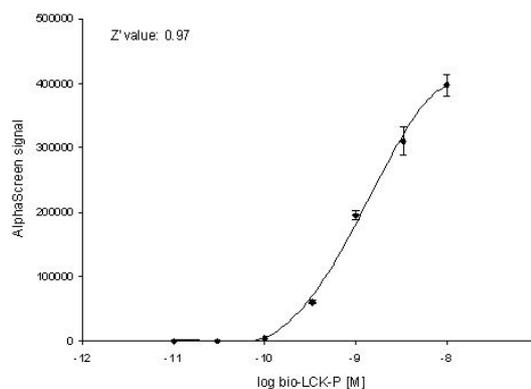


Figure 1: Typical AlphaScreen signal curve using a bio-LCK-P dilution series

To assess the AlphaScreen uniformity, additional microplates containing replicates of 4 nM bio-LCK-P or blanks were measured. The results showed only minimal well-to-well variation, with a CV of 3.3 %, indicating a high signal consistency (Figure 2).

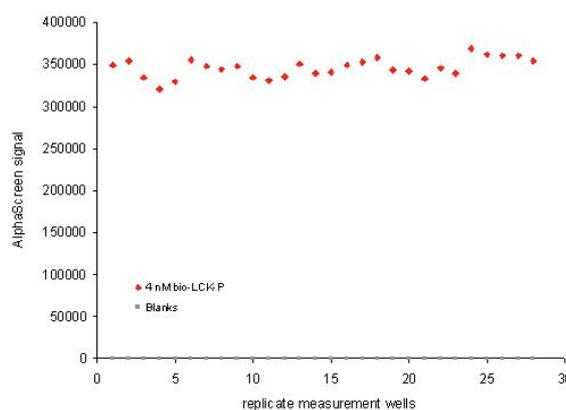


Figure 2: AlphaScreen measurement uniformity using 4 nM bio-LCK-P and blanks.

Conclusion

Taken together, the results summarized in this Application Note demonstrate the excellent performance of the Infinite M1000 PRO for AlphaScreen assays such as the P-Tyr-100 assay. The instrument achieves a detection limit of <100 amol/well, a measurement variation of <3 % (CV) and a high Z' value of 0.97.

The instrument's advanced optics module, in combination with the innovative temperature correction function, enables fast, sensitive and reproducible AlphaScreen measurements. In addition to its well-established multimode reading capacities, the new AlphaScreen/AlphaLISA module makes the Infinite M1000 PRO ideally suited for research, assay development and HTS.

Abbreviations

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) AlphaScreen P-Tyr-100 Assay Kit instructions (PerkinElmer, #6760620)
- 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

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