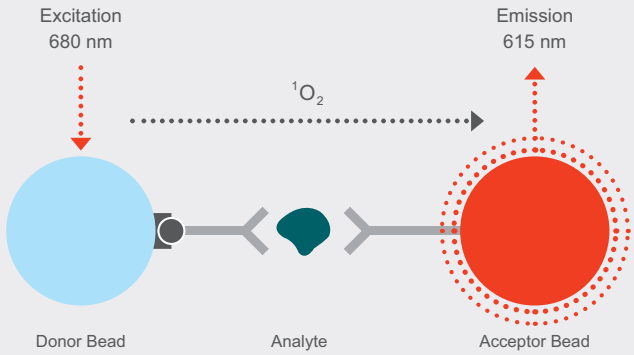

Implementation of AlphaLISA® technology on the Infinite® F200 PRO

Detection of human immunoglobulin G (IgG) using the Infinite F200 PRO's new AlphaLISA function

Introduction

AlphaLISA is a homogeneous, no-wash alternative to conventional ELISAs based on PerkinElmer's bead-based Alpha (Amplified Luminescent Proximity Homogeneous Assay) technology. AlphaLISA assays can be set up as sandwich or competitive immunoassays to detect and quantify molecules of interest in biological samples [1].

High energy excitation of photosensitizer molecules within the AlphaLISA donor beads at 680 nm converts ambient oxygen to singlet oxygen, which in turn is able react with the chemistry in the acceptor beads if these are in close proximity. A cascade of energy transfer steps ultimately results in the generation of a strong luminescence signal at 615 nm, indicating specific binding between the molecules attached to the two bead types. The fluorophores embedded in the AlphaLISA acceptor beads produce a narrower bandwidth signal than the acceptor beads used for classical AlphaScreen® assays.

This makes AlphaLISA assays less prone to signal interference at wavelengths of <600 nm, increasing the sensitivity and robustness of the assay. The use of dedicated AlphaLISA optics permits the analysis of target molecules in blood and serum by drastically reducing the effect of hemoglobin within a sample.

The new module for AlphaScreen and AlphaLISA assays is the latest addition to the Infinite F200 PRO's multimode functionality. In addition to established reading 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® and LanthaScreen™, the Infinite F200 PRO now offers a module for Alpha-based assays, tailored to the needs of low to medium throughput applications and basic research.

The Infinite F200 PRO uses its fluorescence module in combination with a dedicated dichroic mirror and optimized filter sets for AlphaScreen and AlphaLISA applications. While the excitation is performed at 680 nm in both cases, the emission filters are different for AlphaScreen and AlphaLISA readings; AlphaScreen signals are recorded using a 570 (100) nm filter, and AlphaLISA measurements use a 615 (20) nm filter that minimizes hemoglobin-caused assay background.

This Application Note describes the implementation of the AlphaLISA technology onto the Infinite F200 PRO, and describes the use of the reader for the detection of human immunoglobulin G (IgG) using PerkinElmer's AlphaLISA Human IgG Assay Kit [2].

Materials and methods

- AlphaLISA Human IgG Assay Kit, (PerkinElmer, #AL205C)
- White 384-well microplate (Greiner®, #781904)

The AlphaLISA IgG standard was diluted according to the kit protocol and pipetted into replicate wells of a white 384-well microplate; blank wells consisting of assay buffer only (without IgG) were also included. The AlphaLISA acceptor beads and the anti-IgG antibody were diluted according to the assay instructions, added to the samples and incubated for 60 minutes. The donor beads were then diluted appropriately, followed by a further 30 minute incubation period in the dark [2]. The resulting AlphaLISA signal was measured on the Infinite F200 PRO using the measurement settings summarized in Table 1.

Measurement parameters	
Plate definition file (PDFX)	GRE384fw
Excitation filter (bandwidth)	680 (30) nm
Emission filter (bandwidth)	615 (20) nm
Excitation time	1000 ms
Integration time	500 ms
Gain	Calculated from well with the highest signal, or pre-optimized and set manually
Settle time	0 ms

Table 1 AlphaLISA measurement settings

Results

Figure 1 shows a typical IgG standard curve measured with the Infinite F200 PRO in AlphaLISA mode, using a filling volume of 50 µl/well.

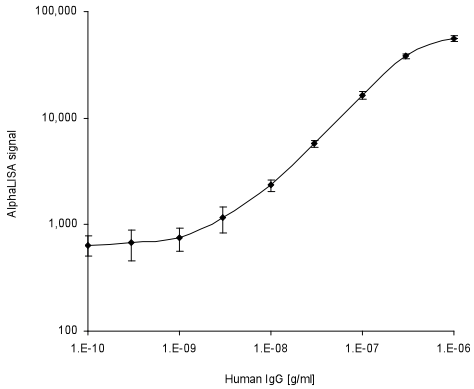

Figure 1 AlphaLISA signal curve – log-log scale

For low concentrations, the curve can be plotted using a linear-linear scale to get a better impression of the measurement linearity, indicated by an R^2 value of 0.999 (Figure 2).

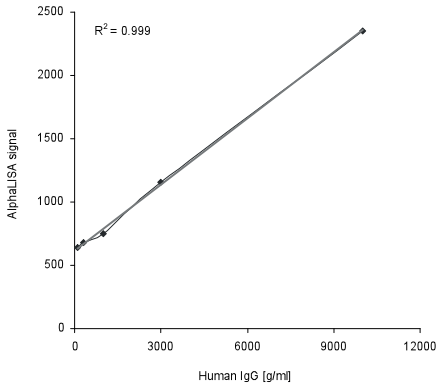

Figure 2 AlphaLISA signal curve – linear-linear scale

The detection limit was calculated by interpolating the average blank signal + 3*stdev of the blank on the IgG standard curve as described in the kit protocol. The Z' value was determined using the following formula:

$$Z'\ value = 1 - \frac{3 * (stdev_{high\ sig} + stdev_{low\ sig})}{(av._{high\ sig} - av._{low\ sig})}$$

stdev $_{high\ sig}$	standard deviation of highest signal
stdev $_{low\ sig}$	standard deviation of lowest signal
av. $_{high\ sig}$	average signal of highest signal
av. $_{low\ sig}$	average signal of lowest signal

The IgG AlphaLISA resulted in a detection limit of 2.2 ng/ml IgG and a Z' value of 0.79.

Conclusion

The results summarized in this Application Note demonstrate the applicability of the Infinite F200 PRO for AlphaLISA-based assays such as the Human IgG Assay. The fluorescence optics-based Alpha module of the instrument delivers reliable measurement results, making the Infinite F200 PRO perfectly suited for sensitive and reproducible AlphaLISA measurements.

Abbreviations

CV	Coefficient of variation
FRET	Fluorescence Resonance Energy Transfer
FP	Fluorescence Polarization
HTRF	Homogeneous Time-Resolved Fluorescence
IgG	Immunoglobulin G
stdev	Standard deviation

References

1) A Practical Guide to Working with AlphaScreen (http://www.urmc.rochester.edu/hts/_source/AlphaScreenPracticalGuide.pdf)
2) AlphaLISA Human IgG Assay Kit Instructions (PerkinElmer, #AL205C) (PerkinElmer, #AL205C)

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