

Implementation of AlphaLISA[®] technology in the Infinite[®] M1000 PRO

Detection of human immunoglobulin G (IgG) using the Infinite M1000 PRO

Introduction

AlphaLISA is a homogeneous, no-wash alternative to conventional ELISA assays based on PerkinElmer's bead-based Alpha (Amplified Luminescent Proximity Homogeneous Assay) technology. AlphaLISAs 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 is able to 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 output 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 AlphaLISAs less prone to signal interference at wavelengths of <600 nm, increasing the sensitivity and robustness of the assay. The no-wash nature of this assay makes it easy to use, and 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 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 applications, using the Infinite M1000 PRO's dedicated luminescence detector 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 AlphaLISA technology on the Infinite M1000 PRO, and its use for the detection of human immunoglobulin G (IgG) using the AlphaLISA IgG Assay Kit [2].

Materials and methods

AlphaLISA Human IgG Assay Kit, (PerkinElmer, #AL205C)
384-well white 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 and added, followed by a further 30 minute incubation in the dark [2]. The resulting AlphaLISA signal was measured on the Infinite M1000 PRO using the measurement settings summarized in Table 1.

Measurement parameters	
Plate definition file	GRE384fw.pdf
Excitation time	100 ms
Integration time	300 ms
Settle time	0 ms
Filter	AlphaLISA
Temperature correction	activated

Table 1 AlphaLISA measurement settings

Results

Figure 1 shows a typical IgG standard curve measured with the Infinite M1000 PRO in AlphaLISA mode, using a filling volume of 50 µl/well in a 384-well plate.

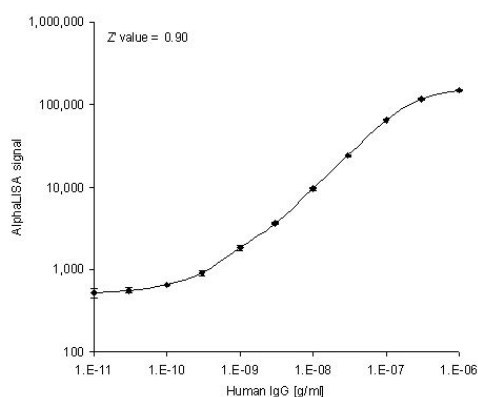


Figure 1 AlphaLISA signal curve – log-log scale

For low concentrations, the curve can be plotted using a linear-linear scale to get a better impression of the excellent measurement linearity, indicated by an R^2 value of 0.9998 (Figure 2).

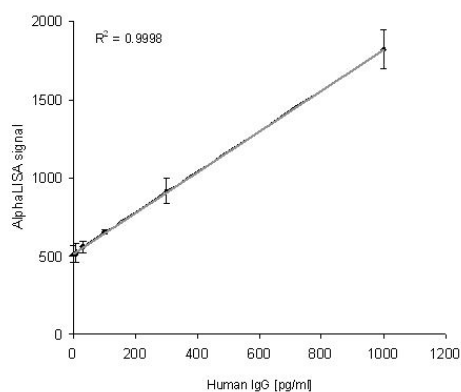


Figure 2 AlphaLISA signal curve – linear-linear scale

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:

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

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 an excellent detection limit of 0.14 ng/ml IgG and a Z' value of 0.9. Furthermore, a very low variation among replicate wells was observed, with CVs below 10 % in all measured IgG dilutions, indicating very good measurement uniformity.

Conclusion

The results summarized in this Application Note demonstrate the excellent performance of the Infinite M1000 PRO for AlphaLISA-based assays such as the human IgG assay. The sensitivity achieved with the Infinite M1000 PRO (0.14 ng/ml) was 3 times better than the detection limit cited by the kit manufacturer (0.45 ng/ml). The instrument's dedicated optics, in combination with its innovative temperature correction function, deliver high quality measurement results, making the Infinite M1000 PRO perfectly suited to robust, 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)

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