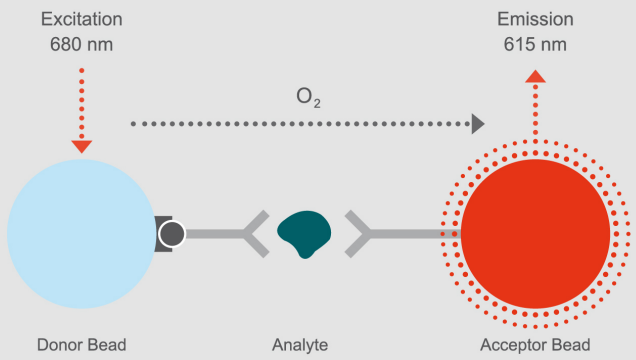

Implementation of AlphaLISA® technology in the Spark™ 10M

Detection of human immunoglobulin G (IgG) using the Spark 10M

Introduction

AlphaLISA is a bead-based, homogeneous, no-wash alternative to conventional ELISAs. Based on 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).

Photosensitizer molecules within the AlphaLISA donor beads are subjected to high energy excitation at a wavelength of 680 nm, which converts ambient oxygen to singlet oxygen. The singlet oxygen then reacts with any acceptor beads in close proximity, causing a cascade of energy transfer steps that ultimately result in the generation of a strong emission 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. Instruments with dedicated AlphaLISA optics permit the analysis of target molecules in blood and serum by drastically reducing the effect of hemoglobin background fluorescence within a sample. In addition, the no-wash nature of this assay makes it easy to use.

Tecan's new multimode reader, the Spark 10M, offers an enhanced detection module for Alpha Technology which uses a high power laser light source for optimal excitation of Alpha donor beads, combined with specific luminescence filters (2) and Tecan's well-established real-time temperature correction function (3). This guarantees ultra-sensitive detection of the emission signal, offering uncompromised performance for AlphaScreen, AlphaLISA and AlphaPlex™ assays.

This application note describes the implementation of the AlphaLISA technology on the Spark 10M and its use for the detection of human immunoglobulin G (IgG) using the AlphaLISA IgG Assay Kit (4).

Conclusion

The results summarized in this application note demonstrate the excellent performance of the Spark 10M for AlphaLISAs, such as the human IgG assay. A sensitivity of 52 pg/ml IgG was achieved, exceeding the 240 pg/ml detection limit cited by the kit manufacturer. The instrument's dedicated optics, in combination with Tecan's well-established temperature correction function (3), deliver high quality measurement results, making the Spark 10M perfectly suited for robust, sensitive and reproducible AlphaLISA measurements.

Abbreviations

Alpha	amplified luminescent proximity homogeneous assay
CV	coefficient of variation
IgG	immunoglobulin G

References

1) A Practical Guide to Working with AlphaScreen http://www.urmc.rochester.edu/hts/_source/alphascreenpracticalguide.pdf
2) Technical Note: Unparalleled flexibility for luminescence detection with the Spark 10M multimode reader. 398597 V1.0. 12-2014.
3) Technical note: New Infinite® M1000 PRO with AlphaScreen module. 396981 V1.0. 11-2011.
4) AlphaLISA Human IgG Assay Kit Instructions (PerkinElmer, #AL205C)

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