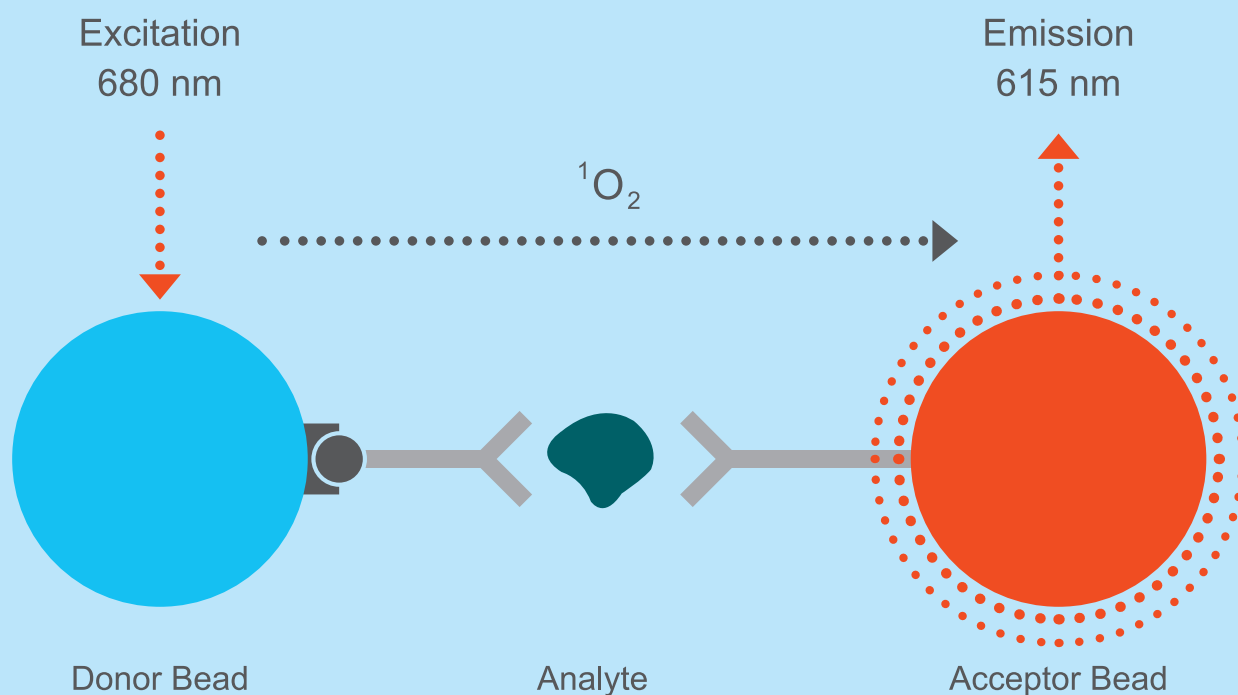


Implementation of AlphaLISA[®]

technology in the Spark[®]

multimode reader.

Application Note



**DETECTION OF HUMAN IMMUNOGLOBULIN G (IGG) USING
THE SPARK MULTIMODE READER PLATFORM**



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.

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 flagship multimode reader platform Spark® 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 and Tecan's well-established real-time temperature correction function. 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 multimode reader and its use for the detection of human immunoglobulin G (IgG) using the AlphaLISA IgG Assay Kit (1).

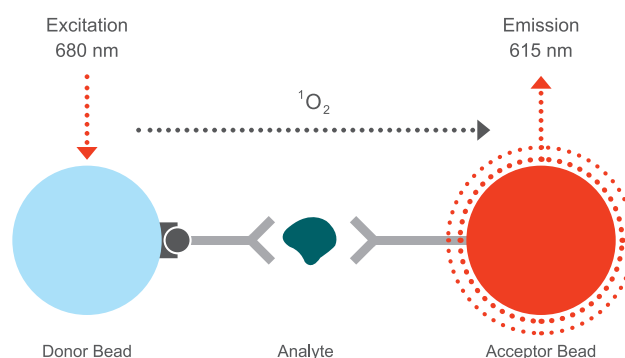


Figure 1: Principle of AlphaLISA assay technology

MATERIALS AND METHODS

- Spark multimode reader (Tecan, Austria)
- AlphaLISA Human IgG Assay Kit (PerkinElmer, USA)
- 384-well white microplates (Greiner Bio-One, Germany)

The AlphaLISA IgG standard was diluted according to the kit protocol and replicates were pipetted into five wells of a white 384-well microplate; blank wells consisting of assay buffer without IgG were also included. The AlphaLISA acceptor beads and the anti-IgG antibody were diluted according to the manufacturer's instructions, added to the samples and incubated for 60 minutes. The donor beads were then diluted appropriately and added to the samples before incubation in the dark for a further 30 minutes. The resulting AlphaLISA signal was measured using the settings in Table 1.

Measurement parameters

Plate definition	GRE384sw
Excitation time	100 msec
Integration time	300 msec
Application	AlphaLISA
Integration wavelength	610-635 nm
Temperature correction	selected
Output	Counts/sec
Settle time	0 msec

Table 1: AlphaLISA measurement settings.

RESULTS

Figure 2 shows a typical IgG standard curve measured with the Spark in AlphaLISA mode using a filling volume of 20 µl/well.





Scatter plot showing the correlation between human IgG concentration (pg/ml) and Alpha ISA signal (counts/sec). The data points show a strong positive linear relationship, with $R^2 = 0.9919$.

human IgG (pg/ml)	Alpha ISA signal (counts/sec)
~100	~2,000
~200	~5,000
~300	~12,000
~1,000	~32,000
~3,000	~82,000

Figure 3: AlphaLISA signal curve – linear-linear scale.

CONCLUSION

The results summarized in this application note demonstrate the excellent performance of the Spark multimode 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, deliver high quality measurement results, making the Spark 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) AlphaLISA Human IgG Assay Kit Instructions
(PerkinElmer, #AL205C)

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