BMG LABTECH

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Increasing throughput with dual emission AlphaLISA assay and simultaneous dual emission detection

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- PHERAstar® *FSX* is an excellent reader for detecting the dual emission AlphaLISA assay
- SDE detection enables users to double their productivity

Introduction

In order to maximize efficiency, screening facilities must constantly strive to increase throughput and minimize sample expenditure, while controlling cost as much as possible. However, saving time, samples and money are irrelevant if in the end the data suffers.

Here we show the performance of the PHERAstar *FSX* in the detection of an AlphaLISA type assay using 2 emission fluorophores. The ability of the PHERAstar *FSX* to perform simultaneous dual emission detection of this assay will allow users to double the information obtained from each well in the same amount of time it takes to run a standard AlphaLISA plate.

Assay Principle

The AlphaLISA principle is quite well characterized and the performance of the PHERAstar series has been described previously in BMG LABTECH Application Note AN260. Recently a modified AlphaLISA has become available that is distinguished by the wavelength of emission with a peak 545 nm as opposed to the traditional 615 nm. Therefore it is now possible to monitor changes in amount of 2 different targets at one time in the same well (Figure 1).
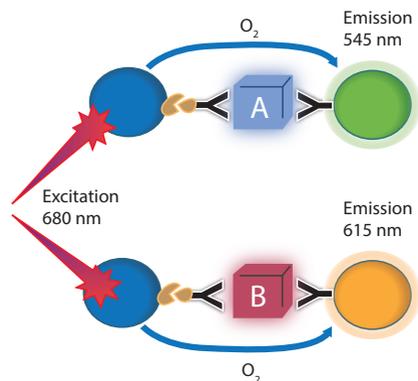
Fig. 1: Dual AlphaLISA principle: Two AlphaLISA's at once For each protein target one antibody was conjugated to an acceptor bead and a 2nd biotinylated antibody was used. Streptavidin coated donor beads were added subsequently.

Both emission wavelengths can be measured simultaneously with the AlphaLISA SDE optic module.

Materials & Methods

- Streptavidin Alpha donor beads
- AlphaLISA® Acceptor beads
- AlphaPLEX™ 545 Acceptor beads
- Purified proteins (Target 1 & 2), Antibodies
- Packard proxiplate F, 384 well, black
- PHERAstar *FSX*

Fig. 2: The PHERAstar *FSX*. The new gold standard for HTS featuring simultaneous dual emission detection.

Antibody pairs were selected for each of two protein targets. For each protein target, one of the paired antibodies was conjugated to either AlphaPLEX™ 545nm acceptor beads (for protein target 1) or AlphaLISA® acceptor beads. The other antibody of each pair was biotinylated.

For test purposes two fold serial dilution of protein target 1 were prepared from a starting concentration of 256 μg/mL. Plates were prepared mixing the varying concentrations with either buffer or 128 μg/mL of target protein 2. A similar dilution series of target protein 2 was prepared and plated with either buffer or 128 μg/mL of target protein 1.

A mixture containing acceptor beads and biotinylated antibodies for both target proteins was prepared and added to the reaction plates. After an incubation period of approximately one hour, a solution of donor beads was added to the reaction plates. Following donor bead addition, reaction plates were incubated for approximately one hour at room temperature before being read on the PHERAstar *FSX* using either a standard AlphaLISA module (Em: 615 nm) or an AlphaLISA SDE module (EmA: 615 nm, EmB: 545 nm).

PHERAstar FSX Instrument settings

Focal height	8.0 mm
Aperture spoon	384 well
Settling time	0.1 sec
Excitation time	0.26 sec
Integration start	0.28 sec
Integration time	0.12 sec

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Results & Discussion

We first felt it important to verify that well characterized AlphaLISA® results would not be compromised when the AlphaLISA® SDE module is used. The performance of 2 modules in the detection of protein target 2 on separate plates is compared in Figure 3 and Table 1.
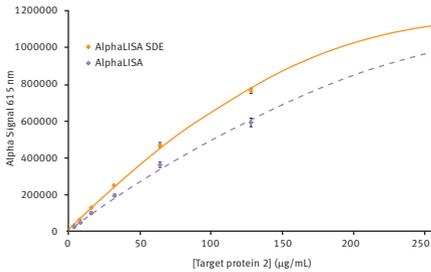
Fig. 3: **Comparison of AlphaLISA and AlphaLISA SDE module performance.** Alpha signal is plotted vs. the different concentrations of target protein 2 present. Average signal from AlphaLISA (◆) AlphaLISA SDE (◆) modules could be plotted using a 2nd order polynomial function with R2 values of 0.9986 and 0.9991 respectively. Error bars indicate standard deviation (n=8).

Table 1: Assay parameters obtained either with the AlphaLISA standard optic module or with the AlphaLISA SDE optic module

	AlphaLISA®	AlphaLISA® SDE
signal/blank*	138.1	124.8
z-prime**	0.797	0.863

*   Signal of protein at 256 µg/ml divided by no protein control
**   based on data for protein at 256 µg/ml as positive control and no protein control as negative control

As can be seen the SDE module exhibits comparable performance to the traditional AlphaLISA® module.

Next we wanted to investigate any effect on detection that might occur if a second signal is present in the well. To that end dilutions of target protein 1 were plated in the presence of target protein 2. Plates were subsequently read with the AlphaLISA SDE module. As can be followed in Figure 4 despite a high level of signal in the 615 channel the detection of signal in the 545 is uncompromised.
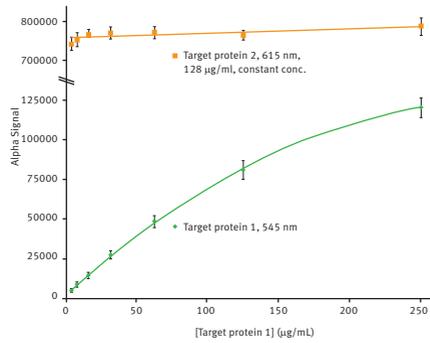
Fig. 4: **Comparison of AlphaLISA SDE module signal detection for 545 nm and 615 nm emission.** Average 545 nm Alpha signal (◆) is plotted vs. the different concentrations of target protein 1 present using a 2nd order polynomial function (R2 = 0.9997). Average 615 nm Alpha signal (■) is plotted for comparison. Error bars indicate standard deviation (n=8).

Similarly we wanted to assess any possible effect that the presence of 545 nm signal may have on the detection in the 615 nm channel. The results in Figure 5 indicate that presence of 545 nm signal does not impact detection of 615 nm signal.
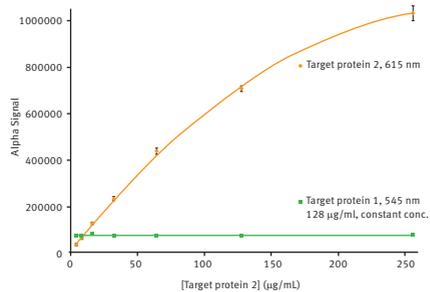
Fig. 5: **Comparison of AlphaLISA SDE module signal detection for 615 nm and 545 nm emission.** Average 615 nm Alpha signal (◆) is plotted vs. the different concentrations of target protein 2 present using a 2nd order polynomial function (R2 = 0.9997). Average 545 nm Alpha signal (■) is plotted for comparison. Error bars indicate standard deviation (n=8).

Conclusion

The PHERAstar *FSX* proves to be an excellent reader to perform detection of Alpha signals at 615 nm and 545 nm simultaneously without compromising data quality.

PHERAstar® *FSX*