ApplicationForum

Increasing throughput with dual emission AlphaLISA® assay and Simultaneous Dual Emission detection

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Introduction

The ability of the microplate reader PHERAstar® FSX to perform Simultaneous Dual Emission detection (SDE) of multiplex AlphaTechnology (AlphaLISA®-AlphaPlex™) allows to double the information obtained from each well in the amount of time a standard AlphaLISA assay is measured.

The possibility to combine AlphaLISA (emission 615 nm) with two new AlphaPlex beads distinguished by their emission peak at either 545 nm or 645 nm, makes it now possible to monitor 2 different targets at once. The PHERAstar FSX can measure simultaneously both AlphaLISA and AlphaPlex emission wavelengths with the AlphaLISA SDE optic module.

Materials & Methods

Antibody pairs were selected for two purified protein targets (t1 & t2). For each target, one paired antibody was conjugated to either AlphaPLEX™ 545 nm acceptor beads (for t1) or AlphaLISA acceptor beads. The second antibody was biotinylated. Two fold serial dilution of t1 were prepared from a concentration of 256 µg/mL. 384-well black plates were prepared mixing the varying concentrations with either buffer or 128 µg/mL of t2. Similar t2 dilution series were prepared and plated with either buffer or 128 µg/mL of t1. A mixture containing acceptor beads and biotinylated antibodies for both targets was added. After one hour incubation, streptavidin Alpha donor beads were added. Plates were incubated for one hour and read on the PHERAstar FSX using either an AlphaLISA (Em: 615 nm) or an AlphaLISA SDE module (EmA: 615 nm, EmB: 545 nm).

Results & Discussion

We first verified that AlphaLISA results would not be compromised by the AlphaLISA SDE module. The performance of 2 modules in the detection of t2 on separate plates was compared (Figure 1).

The SDE module exhibits comparable performance to the traditional AlphaLISA module. Next we investigated any effect on detection that might occur if a second signal is present in the well. t1 dilutions were plated in the presence of t2 and read with the AlphaLISA SDE module. Figure 2 shows that despite a high signal in the 615 channel, 545 signal detection is uncompromised.

Similarly, we assessed no interference of the 545 nm signal on the detection in the 615 nm channel (data not shown). The PHERAstar FSX proves to be an excellent reader for the simultaneous detection of 615 nm and 545 nm Alpha signals without compromising data quality.

Figure 1. AlphaLISA and AlphaLISA SDE module performance comparison. Signal is plotted vs. concentrations of t2. Average signals from AlphaLISA and AlphaLISA SDE modules are plotted using a 2nd order polynomial function (R^2 0.9986 and 0.9991 respectively). Error bars indicate standard deviation.

Figure 2. 545/615 nm comparison of AlphaLISA SDE module signal detection. 545 nm Alpha signal is plotted vs. concentrations of t1 using a 2nd order polynomial function (R^2 0.9997). 615 nm Alpha signal is plotted for comparison. Error bars indicate standard deviation.