Comparison of LanthaScreen™ TR-FRET Assay Performance When Measured Using PMT- or CCD-Based Fluorescence Detection, and Laser or Flash Lamp Excitation Light Source

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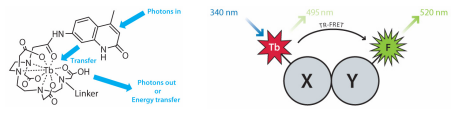
Introduction

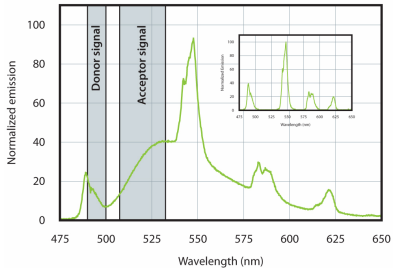
LanthaScreen™ TR-FRET assays using terbium lanthanide chelates as donor fluor molecules paired with fluorescein or GFP as acceptor fluor molecules are widely used to perform enzymatic assays (e.g. kinase, phosphatase, ligase), molecular interactions and metabolite quantification measurements. While this assay platform can be readily measured on most fluorescence detectors equipped with time-resolved features, assay performance may vary greatly based on differences with instrument configuration (i.e. excitation light source, optical components and fluorescence emission detector).

LanthaScreen™ TR-FRET assay performance was evaluated on a PMT-based fluorescence microplate reader (EnVision™) using the standard flash lamp and an available 337nm laser to enhance assay performance. Several dichroic mirrors were compared on this instrument in conjunction with an optimized emission filter set. Further comparisons were performed using both 384- and 1536-well microplate formats and a CCD-based fluorescence microplate imager (ViewLux™) for applications in ultra high-throughput screening.

Figure 1. Principle of LanthaScreen™ TR-FRET Assay

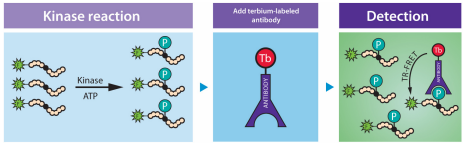


The LanthaScreen™ assay format is based on the use of a long-lifetime terbium chelate as the donor species and fluorescein as the acceptor species. When terbium and fluorescein labeled molecules are brought into proximity, energy transfer takes place, which can be read in a time-resolved manner to reduce assay interference and increase data quality.



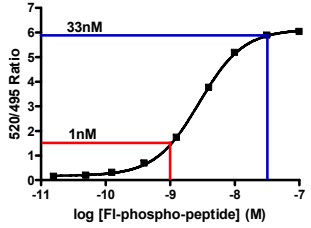
The time-resolved spectra illustrates energy transfer occurring when terbium and fluorescein are brought into proximity via biomolecular interactions. The TR-FRET value is determined as a ratio of the FRET-specific signal measured with a 520 nm filter to that of the signal measured with a 495 nm filter, which is specific to terbium. The inset shows the time-resolved spectra in the absence of energy transfer.

Figure 2. LanthaScreen™ TR-FRET Kinase Assay



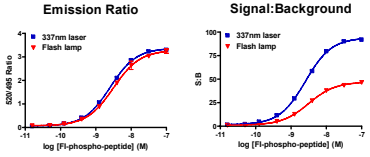
A fluorescein-labeled kinase substrate peptide is incubated with kinase and ATP. Terbium-labeled antibody is then added and phosphorylation is detected by an increase in the TR-FRET ratio. Because the substrate is directly labeled, there is no need to add an acceptor fluor-labeled streptavidin.

Figure 3. Control Phospho-peptide Titration Curve



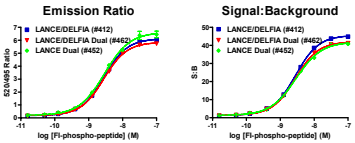
LanthaScreen™ Tb-PY20 Antibody Kit (Invitrogen, PV3552) was used to evaluate the performance of the LanthaScreen™ TR-FRET assay platform using different microplate reader conditions. In brief, 2nM of Tb-PY20 antibody was incubated at RT for 1 hour with a 3-fold serial dilution of Fl-phospho-peptide (15.2pM - 100nM) in TR-FRET dilution buffer. The assay was performed in triplicate in a 384-well black half-volume plate for a final assay volume of 20µl. The plate was read on the EnVision™ using the following recommended filters: 340/30nm excitation filter for flash lamp measurement (Invitrogen, PV00215), 495/10nm and 520/25nm emission filter set (Invitrogen, PV00315); and on the ViewLux™ using the following: 340/60nm excitation filter (PerkinElmer, 14000101), 495/10nm and 520/25nm emission filter set (Chroma, PV003, custom 60mm diameter). Graphs and EC_{50} values were produced using GraphPad Prism®. Signal to background (S:B), percent CV and Z' values were calculated for 1nM and 33nM Fl-phospho-peptide concentrations.

Figure 4. Laser vs Flash Lamp Excitation Light Source



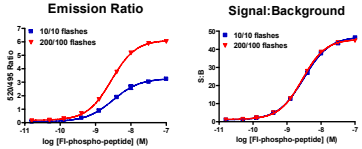
An EnVision™ equipped with an optional 337nm laser was used for the comparison. Ten (10) flashes for 520nm and 495nm emissions were used for both laser and flash lamp measurements. The 337nm laser measurement typically uses a LANCE/DELFIA Dual/Bias dichroic (barcode #446), and we used the LANCE/DELFIA dichroic (barcode #412) for flash lamp measurements.

Figure 5. Dichroic Mirror Comparison



Three available dichroic mirrors were compared: LANCE/DELFIA dichroic (barcode #412); LANCE/DELFIA Dual dichroic (barcode #462) and LANCE Dual dichroic (barcode #452). Two hundred (200) flashes for 520nm emission and 100 flashes for 495nm emission were used for the flash lamp measurements.

Figure 6. Number of Flashes



Comparison of low and high number of flashes: 10 flashes for 520nm and 495nm emission measurements, and 200 flashes for 520nm emission and 100 flashes for 495nm emission. Flash lamp measurements were made with the LANCE/DELFIA dichroic (barcode #412).

Figure 7. Assay Miniaturization

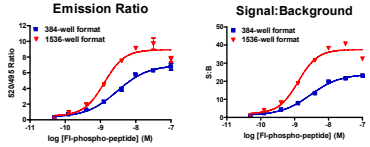
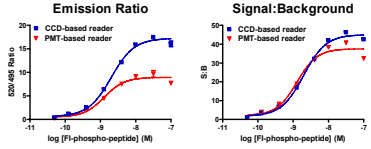


Plate formats and assay volumes were compared using the EnVision™ (flash lamp, LANCE/DELFIA Dual dichroic (barcode #462) and 200/100 flashes). 20µl assay volume in a 384-well black half-volume plate was used for 384-well format and 10µl assay volume in a 1536-well black plate for 1536-well format.

Figure 8. PMT-based vs CCD-based Detection



Using the 1536-well format described above, a CCD-based reader (ViewLux™) was compared to a PMT-based reader (EnVision™ with flash lamp, LANCE/DELFIA Dual dichroic (barcode #462) and 200/100 flashes).

Figure 9. Summary of Test Results

Table 1	EC_{50} (nM)	1nM Fl-phospho-peptide			33nM Fl-phospho-peptide		
		S:B	%cv	Z'	S:B	%cv	Z'
337nm laser	2.55	29	1.0	0.97	90	1.4	0.95
FL #412 (10/10)	3.14	13	2.0	0.88	43	3.0	0.89
FL #412 (200/100)	2.81	13	1.8	0.93	44	2.0	0.94
FL #462 (200/100)	2.77	12	4.2	0.85	41	2.4	0.92
FL #452 (200/100)	2.89	12	5.1	0.79	40	8.5	0.73

This table summarize the results from figures 4, 5 and 6 using the EnVision™. Assay performance was assessed using the calculated EC_{50}, S:B, %cv and Z' values. FL: Flash lamp; #412: LANCE/DELFIA dichroic (barcode #412); #462: LANCE/DELFIA Dual dichroic (barcode #462); #452: LANCE Dual dichroic (barcode #452); 10/10: 10 flashes for 520nm emission readout and 10 flashes for 495nm emission readout; 200/100: 200 flashes for 520nm emission readout and 100 flashes for 495nm emission readout.

Table 2	EC_{50} (nM)	1nM Fl-phospho-peptide			33nM Fl-phospho-peptide		
		S:B	%cv	Z'	S:B	%cv	Z'
384 PMT	2.93	8	3.5	0.83	22	2.2	0.92
1536 PMT	1.28	19	3.1	0.87	38	7.2	0.77
1536 CCD	1.93	17	4	0.86	46	2.7	0.91

This table summarize the results from figures 7 and 8 using the EnVision™ (PMT) and ViewLux™ (CCD). Assay performance was compared using the calculated EC_{50}, S:B, %cv and Z' values. 384: 384-well format (20µl reaction); 1536: 1536-well format (10µl reaction).

Conclusions

- The 337nm laser enhances assay performance compared to the flash lamp excitation light source.
- The single emission LANCE/DELFIA dichroic (barcode #412) used in "Dual mode" offers better performance compared to LANCE/DELFIA Dual (barcode #462) or LANCE Dual (barcode #452) dichroics. However, assay performance using all dichroics tested was excellent.
- Reducing the number of flashes to 10 per emission wavelength did not significantly impact assay performance, but increased the throughput by reducing the plate reading time.
- Assay window improves upon miniaturization from 384 to 1536-well format and with CCD-based detection.
- On a properly configured PMT or CCD-based microplate reader, LanthaScreen™ TR-FRET technology provides optimal assay performance for HTS applications.

For more information on LanthaScreen™:

Applications:	http://www.invitrogen.com/LanthaScreen
FAQ:	http://www.invitrogen.com/downloads/LanthaScreen_FAQ.pdf
Instrumentation:	http://www.invitrogen.com/content.cfm?pageid=10515
EnVision™ setup:	http://www.invitrogen.com/downloads/EnVision.pdf
ViewLux™ setup:	http://www.invitrogen.com/downloads/ViewLux.pdf

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