Labrox® Application Note #004

Labrox® multimode plate reader:

Basic TRF measurements with the Labrox multimode reader

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Introduction

In Time Resolved Fluorescence (TRF) a substance is excited using a short pulse of light and the emission signal is measured after a certain delay time. This delay between the sample excitation and the emission light collection allows the short-lived background fluorescence due to reagents and materials to dissipate so that the specific signal can be measured with maximum sensitivity. Europium chelates are amongst the most used fluorochromes in TRF, due to their long lived emitted fluorescence and large Stoke shift, which makes separation of excitation and emission wavelengths very easy (1).

Labrox multimode plate readers are developed for several applications. Versatile and easy to use they include various detection modes, among them Time Resolved Fluorescence (TRF). In this application note, we demonstrate that Labrox readers in TRF mode can measure Europium with accuracy over a wide range of concentrations. We also show that measurements can be performed in both white and black plates.

Figure 1. Labrox multimode plate reader.

Materials

- DELFIA® 1 nmol/L Europium Standard Solution (PerkinElmer B119-100)
- DELFIA® Enhancement Solution (PerkinElmer 1244-104)
- White 96 well plates (PerkinElmer OptiPlate-F™96)
- Black 96 well plates (PerkinElmer OptiPlate-F™96)
- Labrox Multimode plate reader

Methods

A series of Europium (Eu) dilutions was prepared in enhancement solution, ranging from 0,00001 to 1 nM (Table 1).
Three 200µl replicates of each Eu dilution were dispensed into the appropriate wells in both white and black plates and measured. Enhancement solution samples were included as blank samples.

Europium measuring parameters:

excitation filter: 340 nm
emission filter: 616 nm
Delay: 400 µsec
window: 400 µsec
Cycle: 100 flashes
flash energy: high
excitation spot size: 4 mm
emission spot size: 4 mm.

The results were exported to Excel and analyzed.

Results

The results are shown in Tables 1 and 2 and Figures 1 and 2 below.

Table 1. Average RFU values and SD % of the Europium standard curve (0 –1 nM) (measurements made in white plates).

Eu conc. (nM)	RFU	RFU S-B	SD %
0,00001	20404	348	2
0,0001	21664	1608	4
0,001	29370	9314	1
0,01	96629	76573	2
0,1	562251	542195	3
1	1296297	1276241	9

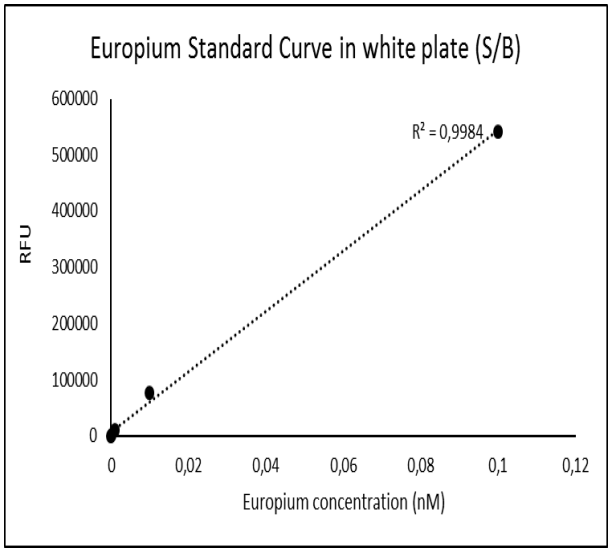

Figure 2. Europium standard curve in white plates. S-B (sample RFU – blank RFU) values were plotted. The calculated correlation coefficient of the curve is 0.9984.

Table 2. Average RFU values and SD % of the Europium standard curve (0 –1 nM) (measurements made in black plates).

Eu concentration (nM)	RFU Value	RFU S-B	SD %
0	3520	0	1
0,00001	3731	211	7
0,0001	4145	625	6
0,001	5659	2139	3
0,01	18970	15450	6
0,1	126818	123298	2
1	782755	779235	1

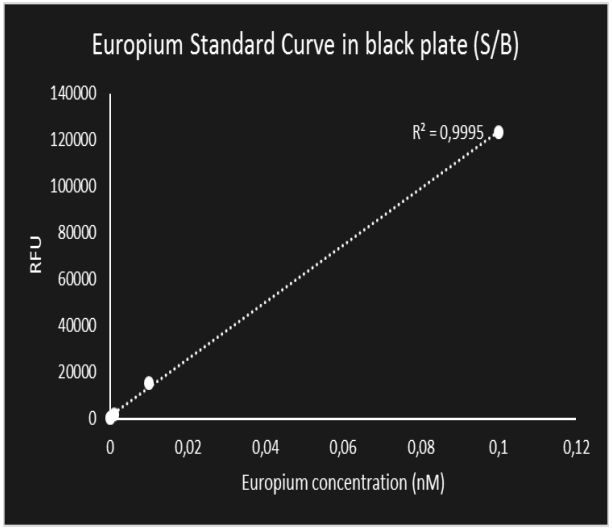

Figure 3. Europium standard curve in black plates. S-B (sample RFU – blank RFU) values were plotted. The calculated correlation coefficient of the curve is 0.9995.

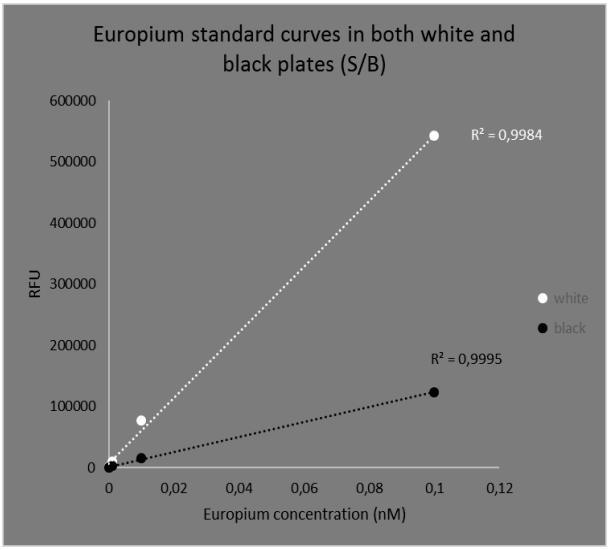

Figure 4. Comparison of the Europium standard curves in white and black plates. S-B (sample RFU – blank RFU) values were plotted.

Conclusions

The linearity obtained in the Europium standard curves shows that the Labrox multimode plate reader TRF mode is effective in measuring accurately Europium in a wide range of concentrations. Europium TRF measurements can be performed both in white and black plates, although the white plates present 1.6-5 times higher RFU values than black plates. In higher Europium concentrations (out of the linear range) this can result in inaccurate measurements in white plates.

References

1. Eleftherius P. Diamandis (1988) “Immunoassays with Time-Resolved Fluorescence Spectroscopy: Principles and Applications”. Clinical Biochemistry, Vol. 21.

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