Labrox® Application Note #002

Labrox® multimode plate reader:

Fluorescein linearity and sensitivity

Susana Laakso

Introduction

Fluorescein label is commonly used by researchers in numerous fluorescence detection methods (e.g flow cytometry and immunofluorescence through conjugation with secondary antibodies, apoptosis detection, nucleotide labelling (1), protein labeling (2), cell labeling (3).

Labrox® multimode plate readers are developed for several applications. Versatile and easy to use they include various detection modes, among them fluorescence (FLU). In this application note, we demonstrate that Labrox readers in FLU mode are appropriate to measure fluorescein with accuracy over a wide range of concentrations.

Figure 1. Labrox multimode plate reader.

Materials

- Fluorescein (PerkinElmer, 100 nmol/l fluorescein standard solution, C557-100)
- Lonza AccuGENE™ Molecular Biology Water cat. BE51200
- Black 96 well plates (PerkinElmer, OptiPlate-F™ 96, 6005270)
- Labrox Multimode plate reader

Methods

A series of fluorescein dilutions was prepared, ranging from 0,001 to 100 nM (Table 1).
Four 100µl replicates of each fluorescein dilution were dispensed into the appropriate wells and measured. Water samples were included as blank samples (controls).

Fluorescein measuring parameters: excitation filter 485 nm with 10 nm bandwith, emission filter 535 nm with 20 nm bandwith. 2000 flashes or 1000 flashes were used, at low energy. The results were exported to Excel and analyzed.

Results

The results are shown in Tables 1 & 2 and Figures 2 & 3 below.

Table 1. Average RFU and SD values of the Fluorescein dilution series (0 –100 nM), measured with 1000 flashes.

1000 flashes			
FITC (nM)	**RFU**	**SD**	**%**
0	2417	39,2	1,6
0,001	2451	39,1	1,6
0,01	3105	101,3	3,3
0,1	16453	173,9	1,1
1	156486	2798,5	1,8
5	705215	8012,2	1,1
10	1358326	11415,8	0,8
50	5277119	101645,4	1,9
100	9756056	399102,1	4,1

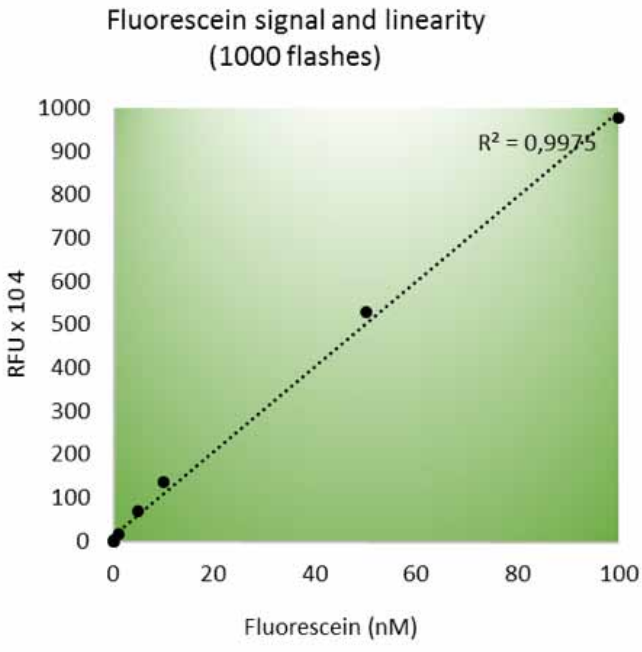

Figure 2. Fluorescein linearity in Labrox multimode plate reader (0 –100 nM) (1000 flashes)

Table 2. Average RFU and SD values of the Fluorescein dilution series (0 –100 nM), measured with 2000 flashes.

2000 flashes			
FITC (nM)	**RFU average**	**SD**	**SD (%)**
0	4773	124	2,6
0,001	5301	232	4,4
0,01	6343	156	2,5
0,1	25798	397	1,5
1	307497	5338	1,7
5	1369631	14897	1,1
10	2466973	31113	1,3
50	9936564	302062	3
100	18099456	819551	4,5

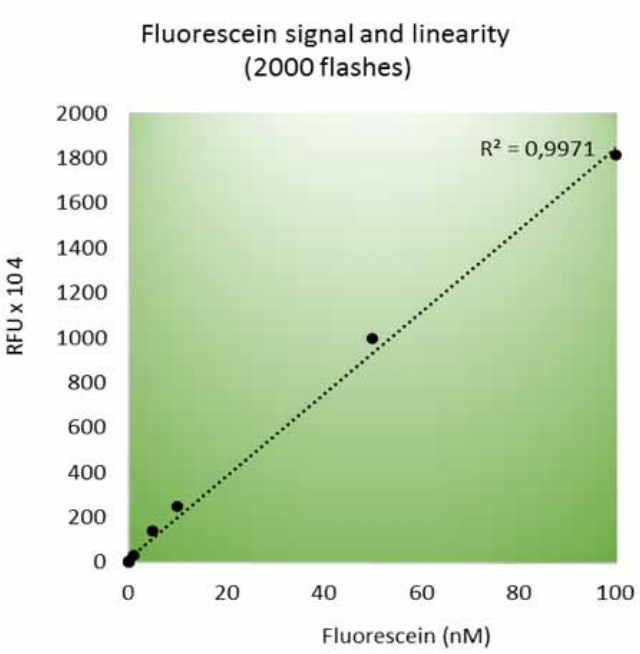

Figure 3. Fluorescein linearity in Labrox multimode plate reader (0 –100 nM) (2000 flashes)

Conclusions

Labrox multimode plate reader is effective in measuring fluorescein concentration. The linearity along the measured dilution series is good, both with 1000 and 2000 flashes, but the signal level increases significantly with a higher number of flashes, allowing the accurate measurement of lower fluorescein concentrations. The results show that Labrox multimode plate reader can be utilized in fluorescein quantitation measurements in a broad range of concentrations and provides reproducible results.

References

1. “FITC/Fluorescein”, Mary Johnson, Labome, MATER METHODS 2011;1:189

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3. “Cell labeling approaches for fluorescence-based in vivo flow cytometry” Costas M. Pitsillides, Judith M. Runnels, Liang Zhi,2 Meixiong Wu, and Charles P. Lin. In: Cytometry A. 2011 Oct; 79(10): 758–765.

Labrox

Rautakatu 5
FIN-20520 Turku, Finland
Tel. +358 (0)50 372 3080

sales@labrox.fi
Twitter: @LabroxCo
www.labrox.fi

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