Labrox
Application
Note #009

Labrox® multimode plate reader:

L-Tryptophan quantification using UV fluorescence measurements on the Labrox multimode reader

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Introduction

L-tryptophan is one of the 22 amino acids that serve as protein building blocks. L-tryptophan is an essential amino acid in the human diet as it cannot be synthesized by the human body, and thus it must be obtained from the food. Furthermore, tryptophan is used as a precursor substance for neuro-hormones, neurotransmitters as well as for vitamins. Due to its essential nature, tryptophan is one of the most investigated amino acids.

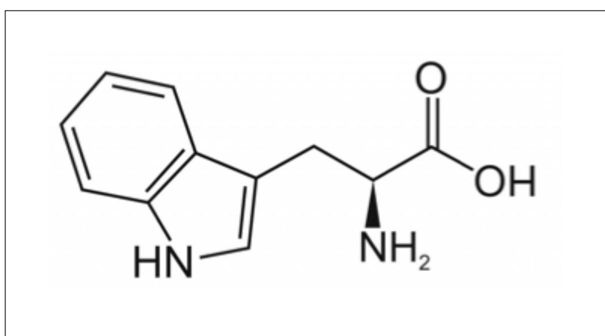

Figure 1. L-Tryptophane structure.

Tryptophan is an important intrinsic fluorescent probe, which can be used to estimate the nature of the microenvironment surrounding tryptophan. Most of the intrinsic fluorescence emissions of a folded protein are due to excitation of tryptophan residues. Moreover, it was observed that the position of tryptophan within the protein affected its fluorescence. In particular, if the aromatic tryptophan residue is present on the surface of the protein, the fluorescence is much higher compared to the fluorescence that can be captured when tryptophan is located inside the protein. Thanks to this observation, fluorescence can be used to determine the conformational state of a protein and its folding process (1, 2).
Labrox multimode plate readers (Fig. 1) are developed for several applications. Versatile and easy to use they include various detection modes, among them Fluorescence Intensity (FI). In this application note, we demonstrate that Labrox readers in FI mode are appropriate to measure L-tryptophan fluorescence.

Figure 2 . Labrox multimode plate reader.

Materials and methods

Materials

- L-tryptophan 99%
  (ACROS Organics, 140590050)
- UV-star microplate, 96-wells, F-bottom, chimney well, µ-clear.
- PBS buffer (Lonza, cat. number BE17-516F)
- Labrox multimode plate reader

Methods:

A 10 µM L-tryptophan solution was prepared in PBS and from this stock solution the following dilution series was prepared for the standard curve measurements (Table 1). PBS buffer was used for all dilutions and as blank.

Table 1. Tryptophan dilution series for the standard curve.

Sample	concentration (µM]
S1	5
S2	2,5
S3	1
S4	0,5
S5	0,1
S6	0,05
S7	0,01
S8	0,005
Blank (PBS)	0

Three replicates (300 µl) of each sample were placed into the microplate and measured.

Measurement parameters:

Method: Fluorescence Intensity, Endpoint mode
Number of flashes: 100
Flash energy: 200 mJ
Excitation filter: 280-12
Dichroic: Trp-LP
Emission filter: 360-20
Z-focus: 8,5 mm

Sensitivity (LoD) calculation:

The LoD was calculated according to IUPAC standards: LoD = 3* SD blank / slope standard curve.

Results

The results obtained are presented on Table 2 and Figure 3.

Table 2. Average signal, standard deviation (SD) and signal to background (S-B) obtained for the different tryptophan concentrations measured.

[Tryptophan] µM	Average	SD	SD %	S-B
0	172404	253	00	
0,005	183264	11594	6	10860
0,01	198658	3459	2	26254
0,05	267212	6285	2	94808
0,1	364579	21144	6	192175
0,5	1033977	11171	1	861573
1	1751812	59489	3	1579408
2,5	3709341	49908	1	3536937
5	6501207	49305	1	6328803

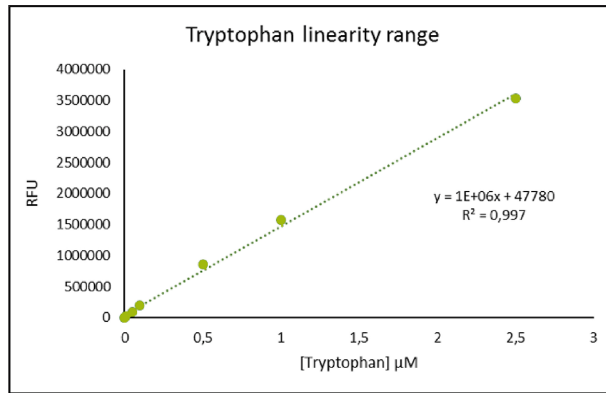

Figure 3. Tryptophan signal linearity.

The standard curve measurement showed a high linearity relation between tryptophan concentration and fluorescence output over a broad concentration range (Fig. 1). The limit of detection was determined to be < 1 nM.

Conclusion

The data shown in this application note demonstrate that Labrox multireader is able to perform tryptophan fluorescence measurements accurately and with very high sensitivity.

References

1. Imoto T, Forster LS, Rupley JA and Tanaka F. (1972) Fluorescence of lysozyme: emissions from tryptophan residues 62 and 108 and energy migration. Proc. Natl. Acad. Sci. USA 69(5): 1151-1155.

2.Vivian JT, Callis PR (2001). “Mechanisms of tryptophan fluorescence shifts in proteins”. Biophys. J. 80 (5): 2093–109

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