

# Photometric Assays with EnVision Multilabel Plate Reader

## Multilabel Detection



### Introduction

Photometric assays are widely used for biochemical or cell based studies. Here we show on examples, NADH and Methyl-red, the possibility of performing those studies using absorbance mode on an EnVision® Multilabel Plate Reader.

NADH is a biomolecule, which participates in a number of biochemical reactions. Therefore it is widely studied among researchers.

Methyl-red is commonly used as a pH indicator. If the pH of the solution is less than 4.2, methyl-red appears as red. If the pH of the solution is above 6.3, it appears as yellow. This indicator can be used to follow, e.g., the bacteria medium growth.

### EnVision in photometric applications

EnVision plate reader is an instrument with modular design, supporting all of the following technologies: AlphaScreen®/AlphaLISA®, luminescence flash and glow, fluorescence intensity, time-resolved fluorescence, fluorescence polarization and absorbance (UV/Visible). Absorbance measurements can be performed in the range of 240-850 nm with a speed of less than 3 min per 1536 well plate.

In absorbance measurements the light is directed through a light guide to the bottom-measuring head where the intensity of the light is measured using a reference photodiode. The light is then directed through the bottom of the plate and focused into the sample. The signal is detected with a photodiode, which is located above the plate.

The absorbance value is calculated by the equation

$$A = -\log(I/I_0)$$

where  $I_0$  is the light intensity without any sample and  $I$  is the intensity after an absorbing or reflecting medium.

## Methods

Dilution series of beta-NADH (Sigma-Aldrich®, N8129) was prepared in TSA, pH 7.4 buffer. The samples were pipetted into a black and clear 1536-well plate (Greiner®, 782096) (7.5 µL/well), 6 replicates. The plate was measured with the EnVision instrument using a photometric filter for 340 nm (PerkinElmer, 2100-5190).

Dilution series of methyl-red was prepared in PBS-buffer. Samples were pipetted into a Greiner® black and clear 1536-well plate (7.5 µL/well), 6 replicates. The plate was measured with the EnVision instrument using a photometric filter for 492 nm (PerkinElmer, 2100-5220).

Dilution series of BSA was prepared into PBS buffer. Samples were pipetted into a 96-well plate (Corning®, 3615), 200 µL/well. The plate was measured with the EnVision instrument using a photometric filter for 280 nm (PerkinElmer, 2100-5350).

## Label properties

Excitation filter	Absorbance 340/492/280
Number of flashes	3 flashes for 340 nm 100 flashes for 492 nm and 280
Number of flashes per A/D	Conversion 1

## Conclusion

In the two examples of NADH and Methyl-red, we show that the EnVision microplate reader is ideally suited to run photometric assays. Its optical range from 240-850 nm and the wide dynamic range of 4 OD combined with its short measurement times provide maximum flexibility, sensitivity and speed.

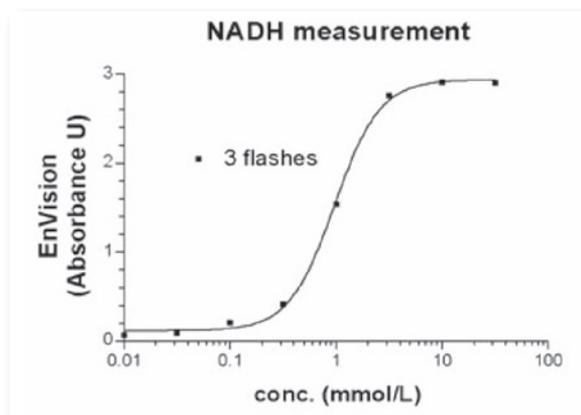


Figure 1. Beta-NADH dilution series measured with EnVision, 3 flashes/well, abs. 340 nm, 1536-well plate.

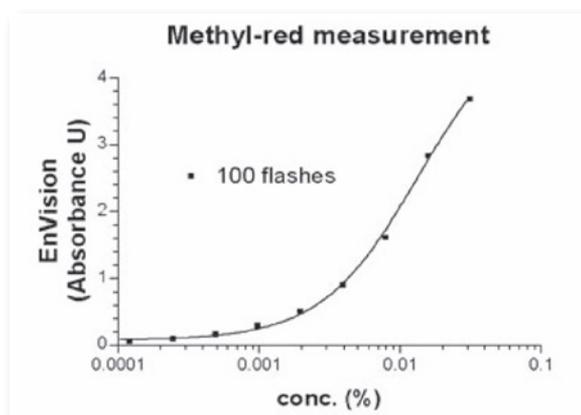


Figure 2. Methyl-red dilution series measured with EnVision, 100 flashes/well, abs. 492 nm, 1536-well plate.

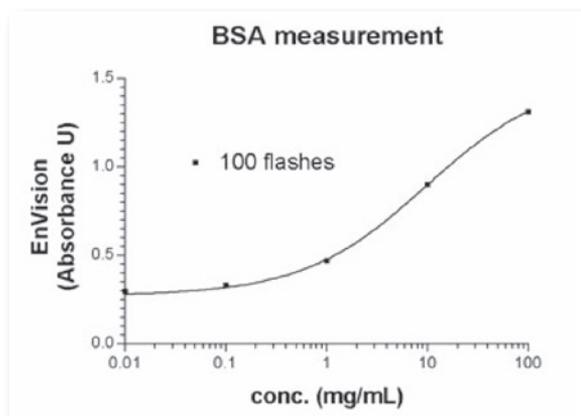


Figure 3. BSA dilution series measured with EnVision, 100 flashes/well, abs. 280 nm, 96-well plate.