Detection of NADH and NADPH with the Omega’s High Speed, Full UV/Vis Absorbance Spectrometer

Application Note 170  Rev. 05/2008

Introduction

Nicotinamide Adenine Dinucleotide (NAD^+), reduced nicotinamide adenine dinucleotide (NADH), nicotinamide adenine dinucleotide phosphate (NADP^+) and reduced nicotinamide adenine dinucleotide phosphate (NADPH) have been known to play vital roles in energy metabolism, antioxidation, and reductive biosynthesis. In recent years, these co-factors have been shown to be involved in other physiological functions as well, including aging, oxidative stress, intracellular calcium homeostasis, ROS production, cell death and gene expression (Figure 1).

Results and Discussion

Enzymatic curves can be easily done that monitor the absorbance change in NADH in any given system. Overlaid NADH absorbance spectra show changes at two wavelengths, 260 and 340 nm (Figure 3).

Materials and Methods

- FLUOstar Omega microplate reader, BMG LABTECH, Offenburg, Germany
- UV Transparent 96 and 384 well Corning microplates [Cat #3635 and #3675].
- NADP and NADPH from Sigma-Aldrich, US
- NAD and NADH from Roth, Germany
- Tris-HCl buffer [pH=7.4]

Changes in NAD^+ or NADPH to NADP^+ can be monitored using absorbance spectroscopy. NADH and NADPH absorb light at 340 nm, where as NAD^+ and NADP^+ do not (Figure 2).

In this application note we show the use of BMG LABTECH’s Omega microplate readers equipped with a UV/Vis absorbance spectrometer for NAD+/NADH or NADP+/NADPH measurements. The easy-to-use data analysis software, MARS, allows for fast determination of co-factor dependent enzymatic activity.
Using MARS evaluation software, linear regression fits were done at two peak wavelengths, 260 and 340 nm. (Figure 4). Note a slope at 340 nm for NADH (green line), but not for NAD\(^+\) (brown line). The 260 nm slope can be used to monitor and to normalize for changes in the concentration of NAD\(^+\) (red line) or NAD\(^+\) (blue line).

**NADP\(^+\)/NADPH**

Enzymatic curves can be done to monitor the absorbance change of NADPH in any given system. A faux conversion curve of NADP\(^+\) to NADPH was produced, keeping the total concentration of dinucleotide constant at 150 \(\mu\)M (Figure 5). Changes in the absorbance spectrum can be seen at two wavelengths, 260 and 340 nm.

Using the MARS evaluation software, linear regression fits of the measurements taken at 260 and 340 nm were done for the NADP\(^+\)/NADPH conversion curve (Figure 6). As expected, there was a linear increase in signal at 340 nm with an increase in NADPH concentration (blue line, \(R^2=0.99\)). However unlike the fit of a usual dose response curve (Figure 4), the linear regression fit at 260 nm showed a slight decrease in signal as the NADPH concentration increased (red line, \(R^2=0.93\)). Theoretically, this peak should have no slope since the concentration of the dinucleotide is kept constant. However, there may be a slight decrease because it seems that NAD\(^+\) absorbs more light at 260 nm than NADH (on Figure 2 compare the 260 nm peaks which have equal concentrations of NAD and NADH). A linear regression fit of the ratioed measurements (340/260 nm) (green line) can be done to correct for this change.

**Limits of Detection for NADH and NADPH**

Using the following equation:

\[
\text{LOD} = 3 \times \text{SD (Blank) / slope (standard curve)}
\]

where the slope is taken from the linear regression fit of the 340 nm absorbance measurements on the NADH and NADPH standard curves (Figure 4 and 6), and the number of blanks measured were 15 or greater.

**Table 1:** LODs of NADH and NADPH in 96 and 384 well plates using 20, 50 and 100 flashes.

<table>
<thead>
<tr>
<th></th>
<th>NADH (µM)</th>
<th>NADH (ng/mL)</th>
<th>NADPH (µM)</th>
<th>NADPH (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>96 well</td>
<td>20</td>
<td>0.748 (497)</td>
<td>1.93 (1610)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>0.580 (386)</td>
<td>1.58 (1322)</td>
<td></td>
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<tr>
<td></td>
<td>100</td>
<td>0.465 (309)</td>
<td>1.11 (931)</td>
<td></td>
</tr>
<tr>
<td>384 well</td>
<td>20</td>
<td>3.761 (2501)</td>
<td>1.80 (1497)</td>
<td></td>
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<tr>
<td></td>
<td>50</td>
<td>3.418 (2773)</td>
<td>1.38 (1151)</td>
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<tr>
<td></td>
<td>100</td>
<td>3.430 (2281)</td>
<td>1.40 (1169)</td>
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</table>

**Conclusion**

The FLUOstar, POLARstar and SPECTROstar Omega microplate readers all have a UV/Vis spectrometer that can measure any absorbance range from 220-850 nm at 1, 2, 5 and 10 nm resolution in under 1 second per well. With this flexibility and speed, absorbance assays can be performed on the Omega that have never been done before on a multidetection microplate reader. Here we show the power of the spectrometer in measuring the cofactors NAD\(^+\), NADH, NADP\(^+\) and NADPH.

**References**