Applications Note

N-Acetylglucosamine Assay

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Introduction:

The N-Acetylglucosamine assay is a rapid colorimetric test used to determine the concentration of N-acetylamino sugars\(^1\). The reaction of the DMAB reagent with an intermediate compound in acidic conditions produces color that can be quantified spectrophotometrically.

Materials:

- PiCOEXPLORER™ Hand-held Spectrophotometer (Cat. No. 0204)
- 0.2 ml Polypropylene Microtubes (Cat. No. 0205 or equivalent)
- Glacial Acetic Acid
- 100mg/ml \(p\)-dimethylaminobenzaldehyde (DMAB) Reagent
  
  \textit{Prepared by mixing 10 g of DMAB in 90ml of glacial acetic acid, 10 ml of HCl, diluted 1:10 in 99\% acetic acid, 1\% HCl}
- 1 mg/ml N-Acetylglucosamine
- 80\(\mu\)M Sodium borate

Methods:

1. Prepare a two-fold serial dilution N-Acetylglucosamine standard curve from 200 \(\mu\)g/ml in a final volume of 100 \(\mu\)l of water in duplicate tubes.
2. Add 100 \(\mu\)l of 80 mM sodium borate to each tube.
3. Vortex briefly and incubate at 100\(^\circ\)C for 10 min.
4. Cool reactions in a 20\(^\circ\)C water bath for 10 min.
5. Add 550 \(\mu\)l of DMAB reagent to each tube and incubate at 37\(^\circ\)C for 20 min.
6. Cool reactions for 15 min in a 20\(^\circ\)C water bath.
7. Dispense 200 \(\mu\)l of each standard into duplicate tubes.
8. Record the absorbance in the Standard Curve mode of the PiCOEXPLORER™ for each calibrator starting with the 0 \(\mu\)g/ml calibrator solution.
9. Select the quadratic calibration curve generated in the Green light mode.

10. Record the absorbance of the sample in the Measurement mode.

Expected Results:

The table below shows typical results for the intensity values and corresponding absorbance values for concentrations ranging from 12.5 to 200. Below the 25 µg/ml the average absorbance was below the recommended minimum of 0.02AU.

<table>
<thead>
<tr>
<th>[N-acetylglucosamine] (µg/ml)</th>
<th>Raw Intensity Values</th>
<th>Calculated Absorbance Values</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>200</td>
<td>35433</td>
<td>34488</td>
</tr>
<tr>
<td>100</td>
<td>42105</td>
<td>42795</td>
</tr>
<tr>
<td>50</td>
<td>44774</td>
<td>47816</td>
</tr>
<tr>
<td>25</td>
<td>52776</td>
<td>49489</td>
</tr>
<tr>
<td>12.5</td>
<td>57465</td>
<td>57412</td>
</tr>
<tr>
<td>0</td>
<td>60208</td>
<td>57835</td>
</tr>
</tbody>
</table>

Reaction mixtures were measured at an LED output level of 10%. Absorbance was calculated using the formula $A = \log(I/I_0)$, where $I$ is the light intensity and $I_0$ is the light intensity of the zero calibrator.

The graph shows the resulting calibrator curve. The error bars for each point are the 95% confidence intervals. The correlation coefficient was 0.9571.

![Graph showing calibration curve for N-acetylglucosamine](image.png)

Figure 1. Calibration curve for N-acetylglucosamine.

References: