Applications Note
Glucosamine Assay

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5 January 2016

Introduction:
The glucosamine assay is a highly specific and sensitive test used for the determination of hexosamines. The reaction of the MBTH reagent with 2,5-anhydroyhexose in acidic solution yields a blue color. Examples of hexosamines used with this assay include glucosamine, galactosamine, and mannosamine.

Materials:
- PiCOEXPLORER™ Hand-held Spectrophotometer (Cat. No. 0204)
- 0.2 ml Polypropylene Microtubes (Cat. No. 0205 or equivalent)
- 1.0 mg/ml Glucosamine
- 5% Sodium nitrite
- 5% Potassium bisulfate
- 12.5% Ammonium sulfamate
- 0.5% 3-Methyl-2-benzothiazolinone hydrazone hydrochloride hydrate (MBTH)
- 0.5% FeCl₃

Methods:
1. Prepare a standard curve of glucosamine from 200, 40, 8, 1.6, and 0 µg/ml in a total volume of 100 µl in duplicate or triplicate.
2. Dispense 100 µl of unknown sample(s) into duplicate or triplicate tubes.
3. Add 10 µl of potassium bisulfate and 10 µl of sodium nitrite to each calibrator and incubate at room temperature for 15 min.
4. Add 10 µl of ammonium sulfamate to each tube and incubate for 5 min with shaking at 800 rpm (Digital Vortex Genie, AthenaES Cat. No. SI-A236).
5. Add 10 µl of 0.5 MBTH to each tube and incubate for 1 h without shaking.
6. Add 10 µl of FeCl₃ to each calibrator and mix well. Incubate for an additional 30 min at room temperature.
7. Record the absorbance in the Standard Curve mode of the PiCOEXPLORER™ for each calibrator starting with the 0 µg/ml calibrator solution.

8. Select the linear calibration curve generated in the Red light mode.

9. Record the absorbance of the unknown sample(s) in the Measurement mode.

Expected Results:

The table below shows typical results. The intensity values and corresponding absorbance values for glucosamine concentrations ranging between 1.6 to 200 µg/ml. Below 8 µg/ml the average absorbance was below the recommended minimum of 0.02AU.

<table>
<thead>
<tr>
<th>[glucosamine] (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>6627</td>
<td>6604</td>
</tr>
<tr>
<td>40</td>
<td>27104</td>
<td>26729</td>
</tr>
<tr>
<td>8</td>
<td>35586</td>
<td>36104</td>
</tr>
<tr>
<td>1.6</td>
<td>38186</td>
<td>38471</td>
</tr>
<tr>
<td>0</td>
<td>39592</td>
<td>38333</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, which was best fitted to a second order polynomial with a correlation coefficient of 0.9999.

![Absorbance vs Glucosamine concentration](image.png)

**Figure 1. Calibration curve for glucosamine.**

References: