Acetate Assay for Cedex Bio and Cedex Bio HT Analyzers

High performance and reproducibility with accurate data

The acetate assay developed for the Cedex Bio and Cedex Bio HT Analyzers provides fast and accurate quantitative measurement of acetate in microbial fermentation. Acetate is converted to acetyl-CoA by acetate kinase (AK) and phosphotransacetylase (PTA). Acetyl-CoA reacts with oxalacetate in presence of citrate synthase (CS) to produce citrate. The oxalacetate for this reaction is generated by conversion of malate by L-malate dehydrogenase (MDH) with the reduction of NAD to NADH. The formation of NADH is measured photometrically by increase in absorbance at 340 nm, which relates to the concentration of acetate in the sample.

\[
\begin{align*}
\text{Acetate} + \text{ATP} & \xrightarrow{\text{AK}} \text{Acetylphosphate} + \text{ADP} \\
\text{Acetylphosphate} + \text{CoA} & \xrightarrow{\text{PTA}} \text{Acetyl-CoA} + \text{Phosphate} \\
\text{Acetyl-CoA} + \text{oxalacetate} + \text{H}_2\text{O} & \xrightarrow{\text{CS}} \text{Citrate} + \text{CoA} \\
\text{L-malate} + \text{NAD}^+ & \xrightarrow{\text{MDH}} \text{oxalacetate} + \text{NADH} + \text{H}^+
\end{align*}
\]

Trust results to make high confidence decisions
- Accurate results over a wide range with automated on-board dilution capability
- Continuous assay development with improved test performance and consistent results
- High comparability with the manual enzymatic photometric assay for acetate determination

![Acetate Assay – Method Comparison](image)

Figure 1: Acetate concentration was determined in an *E. coli* culture using a manual reference test (R-Biopharm, cat. no. 10148261035) and the two assay generations on the Cedex Bio Analyzer in parallel. (Verification data of Roche R&D)

Benefit from a wide and sensitive measuring range

<table>
<thead>
<tr>
<th>Range</th>
<th>Acetate V2 Bio</th>
<th>Acetate V2 Bio HT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetate [mmol/L]</td>
<td>0.25 - 38.9 mmol/L</td>
<td>0.17 - 38.9 mmol/L</td>
</tr>
<tr>
<td>mg/L</td>
<td>15 - 2300 mg/L</td>
<td>10 - 2300 mg/L</td>
</tr>
<tr>
<td>up to 3.89 mol/L</td>
<td>230 g/L with automated on-board dilution</td>
<td></td>
</tr>
</tbody>
</table>

Conserve sample with low volume requirement
- Sample volume of 2μl per test
Save time with improved workflow efficiency
- No sample filtration or pretreatment required
- Ready-to-use reagents
- Long on-board and calibration curve stability
- Barcoded reagents
- Pre- and post-dilution capability

Precision was determined in samples of three concentration levels. Coefficients of variation (CV) were calculated for in-run precision (n=21) and inter-run precision (on 10 days). Representative performance data from Cedex Bio HT Analyzers are shown. Results obtained in individual laboratories may differ.

<table>
<thead>
<tr>
<th>Level</th>
<th>Mean [mmol/L]</th>
<th>CV in-run (%)</th>
<th>CV inter-run (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2.8</td>
<td>1.6</td>
<td>4.5</td>
</tr>
<tr>
<td>2</td>
<td>17.8</td>
<td>1.0</td>
<td>3.9</td>
</tr>
<tr>
<td>3</td>
<td>28.4</td>
<td>0.7</td>
<td>4.0</td>
</tr>
</tbody>
</table>

Ordering information

<table>
<thead>
<tr>
<th>Product</th>
<th>Pack size</th>
<th>Catalog number</th>
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</thead>
<tbody>
<tr>
<td>Acetate Bio V2</td>
<td>4 x 50 tests</td>
<td>07 395 442 001</td>
</tr>
<tr>
<td>Acetate Bio V2 HT</td>
<td>200 tests</td>
<td>07 395 485 001</td>
</tr>
<tr>
<td>Calibrator D Bio</td>
<td>6 x 1 ml</td>
<td>07 368 321 001</td>
</tr>
<tr>
<td>Control D Level 1Bio</td>
<td>6 x 1 ml</td>
<td>07 368 178 001</td>
</tr>
<tr>
<td>Control D Level 2Bio</td>
<td>6 x 1 ml</td>
<td>07 368 186 001</td>
</tr>
<tr>
<td>Control D Level 3Bio</td>
<td>6 x 1 ml</td>
<td>07 368 194 001</td>
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</tbody>
</table>

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Published by
Roche Diagnostics GmbH
Sandhofer Straße 116
68305 Mannheim
Germany

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