Galactose Assay (2nd gen.) for Cedex Bio and Bio HT Analyzers
Improved reagent stability for optimal reliability and convenience

In cell cultures, the galactose concentration in the growth medium has an influence on the glycosylation of recombinant proteins (e.g., therapeutic antibodies), therefore the quality of glycosylated products can be controlled by continuous monitoring of the galactose concentration. Furthermore, galactose can be used instead of glucose as nutrient for the cells. Defined concentrations of galactose can regulate the mitochondrial metabolism, for example, the accumulation of lactate can be reduced.

In yeast fermentation (e.g. with S. cerevisiae), galactose can be a transcription promoter for vectors used in recombinant protein production, and at the same time galactose serves as source of carbon and energy. The addition of galactose to the culture in absence of glucose results in efficient transcription induction. Due to the rapid consumption, the transcription stops once galactose falls under the limit. Therefore a continuous monitoring of the galactose concentration is necessary to enable optimal feeding.

Assay improvements in new generation V2
The new test generation V2 for galactose uses the same reaction principle and results are equal to the former generation. The long-term stability of the reagent formulation has been improved, therefore the on-board time and the calibration interval could be extended, and the test precision is enhanced.

Compared features of the assay generations:

<table>
<thead>
<tr>
<th></th>
<th>1st generation</th>
<th>2nd gen. V2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kit formulation</td>
<td>extra enzyme sol.</td>
<td>ready for use</td>
</tr>
<tr>
<td>On-board stability</td>
<td>4 weeks</td>
<td>4 / 16 weeks (Bio / HT)</td>
</tr>
<tr>
<td>Calibration interval</td>
<td>4 weeks</td>
<td>16 weeks</td>
</tr>
<tr>
<td>Measuring range</td>
<td>10 – 7000 mg/L / 0.06 – 39 mmol/L, up to maximal solubility with auto-dilution</td>
<td>10 – 5000 mg/L / 0.06 – 28 mmol/L, up to maximal solubility with auto-dilution</td>
</tr>
</tbody>
</table>

Figure 1: Comparison of the galactose recovery using the 1st and 2nd generation of the galactose assay on a Cedex Bio HT Analyzer. The results show a perfect equivalence of the test generations and an optimal linearity over the test range.
**Assay principle**

In the Cedex assay, D-galactose is oxidized by nicotinamide adenine dinucleotide (NAD) in presence of β-galactose dehydrogenase (GalDH). The amount of NADH formed in this reaction is measured photometrically at 340 nm and is directly proportional to the amount of D-galactose in the sample.

\[
\text{D-Galactose} + \text{NAD}^+ \xrightarrow{\text{GalDH}} \text{D-Galactonic acid} + \text{NADH} + \text{H}^+
\]

**High specificity**

The galactose assay has no crossreactivity with other carbohydrates in the list below, except of L-arabinose and 2-deoxy-D-galactose, which are not common in cell culture applications.

**Positive signal in the galactose test:**
- D-Galactose, relative recovery = 1.00
- L-Arabinose, relative recovery = 0.47
- 2-Deoxy-D-galactose, relative recovery = 0.27

**No reaction in the galactose test:**
- D-Arabinose
- L-Fucose
- D-Fructose
- L-Galactose
- D-Galactose-6-Phosphate
- D-Galacturonic acid
- D-Glucose
- D-Glucuronic acid
- Lactose

**Precision data**

<table>
<thead>
<tr>
<th>Product</th>
<th>Level 1</th>
<th>Level 2</th>
<th>Level 3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Galactose V2 Bio</td>
<td>126 mg/L / 0.7 mmol/L</td>
<td>1260 mg/L / 7 mmol/L</td>
<td>3780 mg/L / 21 mmol/L</td>
</tr>
<tr>
<td>Galactose V2 Bio HT</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Calibrator D Bio</td>
<td>6 x 1 ml</td>
<td>6 x 1 ml</td>
<td>6 x 1 ml</td>
</tr>
<tr>
<td>Control D Level 1 Bio</td>
<td>6 x 1 ml</td>
<td>6 x 1 ml</td>
<td>6 x 1 ml</td>
</tr>
<tr>
<td>Control D Level 2 Bio</td>
<td>6 x 1 ml</td>
<td>6 x 1 ml</td>
<td>6 x 1 ml</td>
</tr>
<tr>
<td>Control D Level 3 Bio</td>
<td>6 x 1 ml</td>
<td>6 x 1 ml</td>
<td>6 x 1 ml</td>
</tr>
</tbody>
</table>

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

**Ordering information**

<table>
<thead>
<tr>
<th>Product</th>
<th>Pack size</th>
<th>Catalog no.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Galactose V2 Bio</td>
<td>4 x 50 tests</td>
<td>08 391 599 001</td>
</tr>
<tr>
<td>Galactose V2 Bio HT</td>
<td>200 tests</td>
<td>08 391 629 001</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 1 Bio</td>
<td>6 x 1 ml</td>
<td>07 368 178 001</td>
</tr>
<tr>
<td>Control D Level 2 Bio</td>
<td>6 x 1 ml</td>
<td>07 368 186 001</td>
</tr>
<tr>
<td>Control D Level 3 Bio</td>
<td>6 x 1 ml</td>
<td>07 368 194 001</td>
</tr>
</tbody>
</table>

**Regulatory disclaimer**

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