Purification of D-Lactate Dehydrogenase by CIM® DEAE-8 Tube Monolithic Column

Separation Mode: Anion Exchange Chromatography
Instrumentation: Linear Gradient Elution with Peristaltic Pump
Separation Device: CIM® DEAE-8 tube monolithic column

Conditions:
1. Chromatography
   Sample: Crude Lactate Dehydrogenase Solution
   Buffer:
   1. equilibrated with 10 mM phosphate buffer, pH 7.0
   2. washed with 10 mM phosphate buffer, pH 7.0 (35 mL)
   3. washed with 10 mM phosphate buffer, pH 7.0 containing 0.15 M NaCl (50 mL)
   4. eluted by linear gradient of 10 mM phosphate buffer, pH 7.0 containing 0.15 M and 0.5 M NaCl (50 mL each)
   Flow Rate: 1.2 mL/min

2. Assay
   1. Protein: UV at 280 nm
   2. Enzyme Activity: Pyruvic acid-NADH method at 340 nm

Results:

<table>
<thead>
<tr>
<th></th>
<th>Crude Enzyme Solution</th>
<th>Purified Enzyme Solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Volume (mL)</td>
<td>50</td>
<td>12.2</td>
</tr>
<tr>
<td>Total Activity (unit)</td>
<td>4,830</td>
<td>4,160</td>
</tr>
<tr>
<td>Total Protein (mg)</td>
<td>11.2</td>
<td>2.7</td>
</tr>
<tr>
<td>Specific Activity (unit/mg-protein)</td>
<td>431</td>
<td>1,530</td>
</tr>
</tbody>
</table>

Recovery of Enzyme: 86.1 %
Purity of Enzyme: 95 % and over.

Courtesy of Dr. Kimiyasu Isobe, Department of Agrobioscience, Faculty of Agriculture, Iwate University, Japan

CIM® technology is covered by US patents 4889632, 4923610, 4952349 and 5972218. Other patents pending.

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We reserve the right to alter specification details etc. without prior notice or liability!