Pyruvate Assay Kit

Catalog Number KA1674

100 assays

Version: 02

Intended for research use only
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Introduction

Intended Use

Application

✓ Direct Assays: pyruvate in biological samples.
✓ Drug Discovery/Pharmacology: effects of drugs on pyruvate metabolism.

Features

✓ Sensitive and accurate. Use as little as 10 µL samples. Linear detection range in 96-well plate: 2 to 500 µM (17 µg/dL to 4.4 mg/dL) pyruvate for colorimetric assays and 0.2 to 50 µM for fluorimetric assays.
✓ Simple and convenient. The procedure involves addition of a single working reagent and incubation for 30 min at room temperature, compatible for HTS assays.
✓ Improved reagent stability. The optimized formulation has greatly enhanced the reagent and signal stability.

Background

PYRUVATE is a key intermediate in cellular metabolic pathways. Pyruvate can be converted to carbohydrates via gluconeogenesis, to fatty acids or energy through acetyl-CoA, to the amino acid alanine and to ethanol. Abnormal levels of pyruvate have been linked to liver diseases and metabolic disorders. Simple, direct and automation ready procedures for measuring pyruvate concentrations find wide applications in research and drug discovery. Pyruvate Assay Kit uses a single Working Reagent that combines pyruvate oxidase and hydrogen peroxide determination in one step. The color intensity of the reaction product at 570nm or fluorescence intensity at λem/ex = 585/530nm is directly proportional to pyruvate concentration in the sample.
General Information

Materials Supplied

List of component

<table>
<thead>
<tr>
<th>Component</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enzyme Mix</td>
<td>10 mL</td>
</tr>
<tr>
<td>Dye Reagent</td>
<td>120 µL</td>
</tr>
<tr>
<td>Standard</td>
<td>400 µL 25 mM Pyruvate</td>
</tr>
</tbody>
</table>

Storage Instruction

The kit is shipped on dry ice. Store all reagents at -20°C. Shelf life of three months after receipt.

Materials Required but Not Supplied

- Pipeting devices
- Centrifuge tubes
- Clear flat bottom 96-well plates, black 96-well or 384-well plates (e.g. Corning Costar)
- Plate reader

Precautions for Use

- Precautions
Reagents are for research use only. Normal precautions for laboratory reagents should be exercised while using the reagents.
Assay Protocol

Assay Procedure

- Colorimetric method
  
  Note: SH-group containing reagents (e.g. mercaptoethanol, DTT) may interfere with this assay and should be avoided in sample preparation.

1. Equilibrate all components to room temperature. Prepare a 500 µM Standard Premix by mixing 10 µL of the 25 mM Standard and 490 µL H₂O. Dilute Standard in distilled water as follows.

<table>
<thead>
<tr>
<th>No</th>
<th>Premix + H₂O</th>
<th>Vol (µL)</th>
<th>Pyruvate (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>100 µL + 0µL</td>
<td>100</td>
<td>500</td>
</tr>
<tr>
<td>2</td>
<td>80 µL + 20 µL</td>
<td>100</td>
<td>400</td>
</tr>
<tr>
<td>3</td>
<td>60 µL + 40 µL</td>
<td>100</td>
<td>300</td>
</tr>
<tr>
<td>4</td>
<td>40 µL + 60 µL</td>
<td>100</td>
<td>200</td>
</tr>
<tr>
<td>5</td>
<td>30 µL + 70 µL</td>
<td>100</td>
<td>150</td>
</tr>
<tr>
<td>6</td>
<td>20 µL + 80 µL</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>7</td>
<td>10 µL + 90 µL</td>
<td>100</td>
<td>50</td>
</tr>
<tr>
<td>8</td>
<td>0 µL +100 µL</td>
<td>100</td>
<td>0</td>
</tr>
</tbody>
</table>

Transfer 10 µL standards and 10 µL samples into separate wells of a clear flat bottom 96-well plate.

2. For each reaction well, mix 94 µL Enzyme Mix and 1 µL Dye Reagent in a clean tube. Transfer 90 µL Working Reagent into each assay well. Tap plate to mix. Freeze unused reagents for future use.

3. Incubate 30 min at room temperature. Read optical density at 570nm (550-585nm).
   
   Note: if the Sample OD is higher than the Standard OD at 500 µM, dilute sample in water and repeat the assay. Multiply result by the dilution factor.

- Fluorimetric method
  
  For fluorimetric assays, the linear detection range is 0.2 to 50 µM pyruvate. Dilute the Standards prepared in Colorimetric Procedure 1:10 in H₂O.

1. Transfer 10 µL standards and 10 µL samples into separate wells of a black 96-well plate.

2. Add 90 µL Working Reagent (see Colorimetric Procedure). Tap plate to mix.

3. Incubate 30 min at room temperature and read fluorescence at λₑₓ = 530nm and λₑₘ = 585nm.

4. If assays in 384-well plate are desired, use 5µL Standards and 45 µL Working Reagent.
Data Analysis

Calculation of Results

- Colorimetric method
  Subtract blank OD (water, #8) from the standard OD values and plot the OD against standard concentrations.
  Determine the slope using linear regression fitting. The pyruvate concentration of Sample is calculated as
  \[
  [\text{Pyruvate}] = \frac{\text{OD}_{\text{SAMPLE}} - \text{OD}_{\text{H2O}}}{\text{Slope}} \text{ (µM)}
  \]
  \(\text{OD}_{\text{SAMPLE}}\) and \(\text{OD}_{\text{H2O}}\) are optical density values of the sample and water.
  Conversions: 1mM pyruvate equals 8.7 mg/dL or 87 ppm.

- Fluorimetric method
  The pyruvate concentration of Sample is calculated as
  \[
  [\text{Pyruvate}] = \frac{\text{F}_{\text{SAMPLE}} - \text{F}_{\text{H2O}}}{\text{Slope}} \text{ (µM)}
  \]
Resources

References

