Pyruvate dehydrogenase (PDH) Enzyme Activity Microplate Assay Kit ab109902

Overview

Product name: Pyruvate dehydrogenase (PDH) Enzyme Activity Microplate Assay Kit
Detection method: Colorimetric
Sample type: Cell culture extracts, Tissue Lysate, Purified mitochondria
Assay type: Enzyme activity
Assay time: 3h 30m
Species reactivity: Reacts with: Mouse, Rat, Cow, Human

Product overview

Pyruvate dehydrogenase (PDH) Enzyme Activity Microplate Assay Kit (ab109902) can be used to determine the activity of PDH in a human, bovine, mouse, or rat sample. The PDH enzyme is immunocaptured within the wells of the microplate and activity is determined by following the reduction of NAD+ to NADH, coupled to the reduction of a reporter dye to yield a colored reaction product with an increase in absorbance at 450 nm at room temperature. Included for performance of the activity assay are buffer, detergent, reagent mix, and a 96-well microplate with monoclonal antibody pre-bound to the wells of the plate, allowing for a stream-lined assay.

This assay is optimized for use with whole tissue extract when the amount of total material available for assay is 20-100 µg or more. If using cell extract of cultured cells 1 mg of material is required due to the very low levels of enzyme and reduced levels of mitochondria in the extract.

The PDH complex is relatively fragile and sensitive to detergent. Please follow the sample preparation steps provided in the protocol booklet. Other preparation methods may decrease PDH activity. Our Scientific Support team is happy to answer any questions about sample prep.

Review our Metabolism Assay Guide to learn about assays for metabolites, metabolic enzymes, mitochondrial function, and oxidative stress, and also about how to assay metabolic function in live cells using your plate reader.

Notes

Store 20X Buffer, Detergent, and Microplate at 4°C.
Store 5X Stabilizer at -20°C.
Store 20X Reagent Mix, 100X Reagent Dye and 100X Coupler at -80°C.

Platform

Microplate reader
Properties

Storage instructions
   Please refer to protocols.

<table>
<thead>
<tr>
<th>Components</th>
<th>96 tests</th>
</tr>
</thead>
<tbody>
<tr>
<td>20X Buffer</td>
<td>1 x 15ml</td>
</tr>
<tr>
<td>20X Reagent Mix</td>
<td>2 x 600µl</td>
</tr>
<tr>
<td>5X Stabilizer</td>
<td>1 x 13ml</td>
</tr>
<tr>
<td>96-well Microplate (12 strips)</td>
<td>1 unit</td>
</tr>
<tr>
<td>Coupler</td>
<td>1 x 250µl</td>
</tr>
<tr>
<td>Detergent</td>
<td>2 x 1ml</td>
</tr>
<tr>
<td>Reagent Dye</td>
<td>1 x 250µl</td>
</tr>
</tbody>
</table>

Images

Mitochondria, tissue extracts and whole cultured cell extracts all show linear relationships between signal and sample load at limiting concentrations. The rates shown were determined as change in OD over time, and these are best represented as change in milliOD per minute.

Example of microplate reader recorded data from bovine heart mitochondria (100 µg/well) (top trace) and 2-fold dilutions (stepwise lower traces) using Pyruvate dehydrogenase (PDH) Enzyme Activity Microplate Assay Kit (ab109902).
Schematic diagram showing the reaction involved in Pyruvate dehydrogenase (PDH) Enzyme Activity Microplate Assay Kit (ab109902).

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