Glyceraldehyde 3 Phosphate Dehydrogenase Activity Assay Kit (Colorimetric) ab204732

Overview

Product name: Glyceraldehyde 3 Phosphate Dehydrogenase Activity Assay Kit (Colorimetric)

Detection method: Colorimetric

Sample type: Tissue, Adherent cells, Suspension cells

Assay type: Enzyme activity

Assay time: 0h 20m

Species reactivity: Reacts with: Other species, Mammals

Product overview:
Glyceraldehyde 3 Phosphate Dehydrogenase Activity Assay Kit (Colorimetric) (ab204732) provides a simple and sensitive method for monitoring GAPDH activity in various samples. In this assay, GAPDH catalyzes conversion of GAP into BPG and an intermediate, which reacts with a developer to form a colored product that absorbs maximally at 450 nm. Our high-throughput adaptable assay can detect GAPDH activity as low as 100 µU/mL in a variety of samples.

GAPDH assay protocol summary:
- add samples and standards to wells
- add reaction mix
- analyze with microplate reader for 10 min every 2-3 min

Notes:
GAPDH (Glyceraldehyde-3-Phosphate Dehydrogenase; EC 1.2.1.12) catalyzes the conversion of Glyceraldehyde-3-Phosphate (GAP) to 1, 3-Bisphosphate Glycerate (BPG) and plays a key role in glycolysis. The enzyme is involved in cellular processes such as apoptosis, membrane trafficking, iron metabolism and nuclear translocation. GAPDH (housekeeping gene) expression is stable and constitutive. Deregulation of GAPDH activity is associated with abnormal cell proliferation and carcinogenesis. Accurate quantitation of GAPDH activity is important for diagnosing diseases and studying normal cellular physiology.

Platform: Microplate reader

Properties

Storage instructions: Store at -20°C. Please refer to protocols.
Function
Has both glyceraldehyde-3-phosphate dehydrogenase and nitrosylase activities, thereby playing a role in glycolysis and nuclear functions, respectively. Participates in nuclear events including transcription, RNA transport, DNA replication and apoptosis. Nuclear functions are probably due to the nitrosylase activity that mediates cysteine S-nitrosylation of nuclear target proteins such as SIRT1, HDAC2 and PRKDC (By similarity). Glyceraldehyde-3-phosphate dehydrogenase is a key enzyme in glycolysis that catalyzes the first step of the pathway by converting D-glyceraldehyde 3-phosphate (G3P) into 3-phospho-D-glyceroyl phosphate.

Pathway
Carbohydrate degradation; glycolysis; pyruvate from D-glyceraldehyde 3-phosphate: step 1/5.

Sequence similarities
Belongs to the glyceraldehyde-3-phosphate dehydrogenase family.

Post-translational modifications
S-nitrosylation of Cys-152 leads to interaction with SIAH1, followed by translocation to the nucleus.
ISGylated.

Cellular localization
Cytoplasm > cytosol. Nucleus. Cytoplasm > perinuclear region. Membrane. Translocates to the nucleus following S-nitrosylation and interaction with SIAH1, which contains a nuclear localization signal (By similarity). Postnuclear and Perinuclear regions.

Images
Typical NADH Standard Curve.

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<table>
<thead>
<tr>
<th>Components</th>
<th>100 tests</th>
</tr>
</thead>
<tbody>
<tr>
<td>GAPDH Assay Buffer</td>
<td>1 x 25ml</td>
</tr>
<tr>
<td>GAPDH Developer</td>
<td>1 vial</td>
</tr>
<tr>
<td>GAPDH Positive Control</td>
<td>1 vial</td>
</tr>
<tr>
<td>GAPDH Substrate</td>
<td>1 vial</td>
</tr>
<tr>
<td>NADH Standard (500 nmol)</td>
<td>1 vial</td>
</tr>
</tbody>
</table>
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GAPDH activity in the Positive Control, 3T3 cells lysate and MDA-MB-468 cells lysate.

GAPDH specific activity calculated from 3T3 cell lysate (11.1 µg protein), and MDA-MB-468 cell lysate (2.76 µg protein).

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