Alkaline Phosphatase Assay Kit (Colorimetric) ab83369

Overview

Product name: Alkaline Phosphatase Assay Kit (Colorimetric)
Detection method: Colorimetric
Sample type: Urine, Serum, Plasma, Other biological fluids, Tissue Extracts, Cell Lysate, Cell culture media
Assay type: Enzyme activity (quantitative)
Sensitivity: > 10 µU
Range: 10 µU - 250 µU
Assay time: 1h 10m

Species reactivity: Reacts with: Other species, Mammals

Product overview

Alkaline Phosphatase Assay Kit (Colorimetric) ab83369 is a highly sensitive, simple, direct and HTS-ready colorimetric assay designed to measure alkaline phosphatase (ALP) activity in serum and other mammalian samples.

The kit is a pNPP assay; it uses p-nitrophenyl phosphate (pNPP) as a phosphatase substrate which turns yellow (λmax= 405 nm) when dephosphorylated by ALP.

Alkaline phosphatase assay protocol summary:
- add samples and standards to wells
- add pNPP solution to sample wells (not to standards)
- add ALP enzyme solution to standard wells (not to samples)
- incubate for 60 min at room temp
- add stop solution
- analyze with microplate reader

Notes

This kit contains 10 substrate tablets providing convenience for multiple usages.

pNPP Assays

pNPP assays are a class of phosphatase assays using p-nitrophenyl phosphate (pNPP) as a phosphatase substrate. They can be used to measure alkaline phosphatase, neutral phosphatase, and acid phosphatase activity. These different types of phosphatases are active respectively in alkaline, neutral, and acid assay buffers. pNPP assays include Alkaline Phosphatase Assay Kit ab83369 and Acid Phosphatase Assay Kit ab83367.

Platform

Microplate reader

Properties
Storage instructions
Store at -20°C. Please refer to protocols.

<table>
<thead>
<tr>
<th>Components</th>
<th>Identifier</th>
<th>500 tests</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALP Assay buffer</td>
<td>NM</td>
<td>1 x 100ml</td>
</tr>
<tr>
<td>ALP Enzyme (Lyophilised)</td>
<td>Green</td>
<td>1 vial</td>
</tr>
<tr>
<td>pNPP Substrate (10 tablets)</td>
<td>Red</td>
<td>1 vial</td>
</tr>
<tr>
<td>Stop Solution</td>
<td>WM</td>
<td>1 x 10ml</td>
</tr>
</tbody>
</table>

Relevance
Alkaline phosphatase (ALP) catalyzes the hydrolysis of phosphate esters in alkaline buffer and produces an organic radical and inorganic phosphate. Changes in alkaline phosphatase level and activity are associated with various disease states in the liver and bone.

Cellular localization
Cell membrane; Lipid-anchor, GPI-anchor.

Images

Alkaline phosphatase was measured in Caco-2 cells after 48 hours treatment with PMA and Butyrate using Alkaline Phosphatase assay kit (ab83369).

Standard curve: mean of duplicates (+/- SD) with background reads subtracted
ALP measured in cell culture supernatants showing activity (U) per L of tested sample. Samples were diluted 1-16 fold.

ALP measured in biological fluids showing activity (U) per L of tested sample. Samples were diluted 4-64 fold.

Measurement of ALP activity using ab83369. a. ALP activity in fresh medium (80 μL, without culturing), 3 day old HeLa cell culture medium (80 μL) and human serum (80 μL, 1/10 dilution). b. ALP activity in HeLa cells. 5 x 10⁴ HeLa cells were homogenized in 1 mL of Assay Buffer, diluted 1/10 in Assay Buffer, and 80 μL used for the measurement.

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