Name: Colorimetric Alkaline Phosphatase Assay Kit, Yellow Color
Catalog Number: FP-JQ6720  500 assays
Components:
- Component A: pNPP (light sensitive)  1 vial
- Component B: Assay buffer  1 bottle (25 mL)
- Component C: Alkaline phosphatase standard  1 vial (lyophilized powder, 10 units)
Storage: Keep in freezer. Protect from light and moisture

Introduction
Alkaline phosphatase is a highly sensitive enzyme for ELISA, immuno-histochemical, Northern, Southern and Western blot applications. It is widely used in various biological assays (in particular, immunoassays) and ELISA-based diagnostics. This Alkaline Phosphatase Assay Kit uses pNPP, a chromogenic phosphatase substrate, to quantify alkaline phosphatase activity in solutions, in cell extracts as well as on solid surfaces (such as PVDF membranes). The kit provides all the essential components with our optimized 'mix and read' assay protocol that is compatible with HTS liquid handling instruments.

This Alkaline Phosphatase Assay Kit can be performed in a convenient 96-well or 384-well microtiter-plate format and easily adapted to automation with no separation steps required. Its signal can be easily read by absorbance microplate reader around 400 nm.

Kit Key Features
- Optimized: Optimized conditions for detecting alkaline phosphatase activity.
- Continuous: Easily adapted to automation with no separation required.
- Convenient: Formulated to have minimal hands-on time. No wash is required.
- Non-Radioactive: No special requirements for waste treatment.
Directions for use

Protocol for one 96-well plate

Brief Summary

Prepare assay reaction mixture (50 μL) → Add alkaline phosphatase standards or test samples (50 μL) → Incubate at RT or 37°C for 5-30 min → Read absorbance at 400 nm

Note: Thaw all the kit components to room temperature before starting your experiment.

Note: Unless otherwise noted, all unused stock solutions should be divided into single-use aliquots and stored at -20 °C after preparation. Avoid repeated freeze-thaw cycles.

1. Prepare pNPP stock solutions:
   1.1 pNPP stock solution (100X): Add 300 μL of distilled H2O into the vial of pNPP (Component A). Mix the reagents well. The stock solution should be used promptly. Any remaining solution need be aliquoted and refrozen at -20°C.
   
   Note: It will be good for 3-4 weeks if stored at -20°C.

2. Prepare pNPP reaction mixture:
   2.1 Add 100 μL of distilled H2O with 0.1% BSA (H2O - 0.1% BSA) to Alkaline Phosphatase Standard (Component C, 10 units) to generate a 100 units/mL Alkaline Phosphatase standard solution.

3. Prepare serial alkaline phosphatase (0 to 100 mU/mL) solutions:

   Add 10 μL of 100 units/mL Alkaline Phosphatase standard solution to 990 μL of H2O - 0.1% BSA to generate a 1,000 mU/mL Alkaline Phosphatase standard solution. Then take 100 μL of 1,000 mU/mL Alkaline Phosphatase standard solution to perform a 1:10 dilution to obtain 100 mU/mL Alkaline Phosphatase standard solution (AS7). Then perform 1:3 serial dilution to obtain remaining standards (AS6 - AS1). Note: The unused portion of diluted alkaline phosphatase standard solution should be discarded.

Table 1. Layout of Alkaline phosphatase standards and samples in a white/clear bottom 96-well microplate:

| BL | BL | TS | TS | ..... | ..... |
| AS1 | AS1 | ..... | ..... | ..... | ..... |
| AS2 | AS2 | ..... | ..... | ..... | ..... |
| AS3 | AS3 | ..... | ..... | ..... | ..... |
| AS4 | AS4 | ..... | ..... | ..... | ..... |
| AS5 | AS5 | ..... | ..... | ..... | ..... |
| AS6 | AS6 | ..... | ..... | ..... | ..... |
| AS7 | AS7 | ..... | ..... | ..... | ..... |

Note: AS = Alkaline Phosphatase Standards, BL=Blank Control, TS=Test Samples.

Table 2. Reagent composition for each well:

<table>
<thead>
<tr>
<th>Well</th>
<th>Volume</th>
<th>Reagent</th>
</tr>
</thead>
<tbody>
<tr>
<td>AS1 - AS7</td>
<td>50 μL</td>
<td>Serial Dilution (0.1 to 100 mU/mL)</td>
</tr>
<tr>
<td>BL</td>
<td>50 μL</td>
<td>H2O - 0.1% BSA</td>
</tr>
<tr>
<td>TS</td>
<td>50 μL</td>
<td>test sample</td>
</tr>
</tbody>
</table>
4. Preparation of working solutions
Add 50 μL of pNPP Stock solution (100X) into 5 mL of Assay Buffer (Component B) to make a total volume of 5.05 mL of pNPP working solution.

5. Run alkaline phosphatase assay in supernatants:
   5.1 Prepare alkaline phosphate standards (AS), blank controls (BL), and test samples (TS) according to the layout provided in Tables 1 and 2. For a 384-well plate, use 25 μL of reagent per well instead of 50 μL.
   5.2 Add 50 μL pNPP working solution to each well of alkaline phosphate standard, blank control, and test samples to make the total alkaline phosphate assay volume of 100 μL/well. For a 384-well plate, add 25 μL of pNPP working solution into each well instead, for a total volume of 50 μL/well.
   5.3 Incubate the reaction at the desired temperature for 10 to 30 minutes, protected from light.
   5.4 Monitor the absorbance increase at 400 nm using an absorbance plate reader.

6. Run alkaline phosphatase assay in cells:
   6.1 Treat your cells as desired.
   6.2 Add equal volume of pNPP working solution into each cell well (such as 100µL/96-well plate or 50 μL/384-well plate).
   Note: Alternatively, remove the growth medium from the cell plate, and make 1:1 dilution of the 5 mL working solution with 5 mL distilled H 2 O. Then add 100 μL (for a 96-well plate) or 50 uL (for a 384-well plate) of 1:1 diluted working solution to the cell wells.
   6.3 Incubate the reaction for 30 to 60 minutes at the desired temperature, protected from light.
   6.4 Monitor the absorbance increase with an absorbance plate reader at 400 nm.

Data Analysis:
The reading (Absorbance) obtained from the blank standard well is used as a negative control. Subtract this value from the other standards’ readings to obtain the base-line corrected values. Then, plot the standards’ readings to obtain a standard curve and equation. This equation can be used to calculate ALP samples. The typical data are shown in Figure 1 (alkaline phosphatase standard curve).

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The reading (Absorbance) obtained from the blank standard well is used as a negative control. Subtract this value from the other standards’ readings to obtain the base-line corrected values. Then, plot the standards’ readings to obtain a standard curve and equation. This equation can be used to calculate ALP samples. The typical data are shown in Figure 1 (alkaline phosphatase standard curve).

Figure 1. Alkaline phosphatase dose response was measured with the Colorimetric Alkaline Phosphatase Assay Kit in a white/clear bottom 96-well plate using a NovoStar microplate reader (BMG Labtech).
References


Technical and scientific information

Related products

- pNPP tablets, 732500
- FDP, FP-72573A
- Fluorimetric Alkaline Phosphatase Assay Kit, Blue, JQ6730
- Fluorimetric Alkaline Phosphatase Assay Kit, Green, JQ6740
- Fluorimetric Alkaline Phosphatase Assay Kit, Red, JQ6750

Ordering information

Catalog size quantities and prices may be found at www.interchim.com/
Please inquire for higher quantities (availability, shipment conditions).

For any information, please ask: FluoProbes® / Interchim; Hotline: +33(0)4 70 03 73 06

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