

pNPP

CHROMOGENIC SUBSTRATES

ELISA AP Substrate

Cat. no. 4400

Product Characteristics

pNPP (p-nitrophenylphosphate) is a chromogenic substrate for Alkaline Phosphatase (AP). In the presence of AP, pNPP is hydrolyzed rapidly to p-nitrophenol and inorganic phosphate. Liberated p-nitrophenol is seen as a clear yellow color that is measured at 405/620 nm.

pNPP, liquid ready-to-use substrate, is highly active compared to pNPP ONE (cat. No. 4401). The substrate gives superior results due to its sensitivity, allowing very low detection limits.

Composition & Properties

pNPP is a liquid, ready-to-use and aqueous formulation. The buffer solution contains stabilized pNPP and appropriate stabilizing components. Recommended stop solution is an equal volume of 0.1 M sodium hydroxide solution.

Working Procedure

1. The desired amount of substrate is poured into a sealed container and allowed to reach room temperature in the dark.
2. Recommended volumes are 100 µl substrate solution per microtiter well. Development time is typically 15-30 minutes. Optimal time is determined by the user and will vary according to the procedure and the incubation temperature.
3. 100 µl of 0.1 M NaOH is then added and mixed thoroughly.
4. In kinetic assays, the color is read directly at 405 nm. For endpoint assays the absorbance is read at 405/620 nm.

Tips & Tricks

- For use in hot climate countries, due to superior background characteristics, we recommend pNPP ONE (cat. no. 4401)
- A stop solution of 0.1 M NaOH requires no hazardous labelling. In case a higher assay sensitivity is required, a 1.0 M NaOH may be used as stop solution offering an approx. increase of OD of 3%. In both cases a 1 hour stop stability is guaranteed.

Handling & Storage

- Store solution at 2-8 °C in the dark.
- Avoid exposure to light and heat.
- Re-dispense only into bottles made of High Density Polyethylene (HDPE), amber color. Dispensing guidelines are available upon request.



Our Immunoassay Solutions are eco-friendly, creating a healthy work environment and preserving natural resources, while helping our customers fulfill significant regulatory requirements. **We call it ECO-TEK.**