

pNPP ONE

CHROMOGENIC SUBSTRATES

ELISA AP Substrate

Cat. no. 4401

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 ONE liquid, ready-to-use substrate is an active and stable substrate formulation. pNPP ONE shows superior background characteristics due to its high temperature robustness, compared to pNPP (cat. no. 4400) making it suitable for use in hot climate countries.

Composition & Properties

pNPP ONE 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

- If a more sensitive substrate giving lower detection limits is needed, we recommend pNPP (cat no. 4400). This substrate offers a 10% higher sensitivity than pNPP ONE.
- 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.**