

PNPP – Phosphatase Substrate

34045 34047 34064 37620

0338.4

Number	Description
37620	Phosphatase Substrate Kit Kit Contents: Diethanolamine Substrate Buffer (5X) (5.1M diethanolamine, pH 9.8), 225mL PNPP Tablets , 105 tablets, each tablet contains 5mg of <i>p</i> -nitrophenyl phosphate disodium salt with a balance of inert filler
34064	Diethanolamine Substrate Buffer (5X) (5.1M diethanolamine, pH 9.8), 225mL
34047	PNPP Tablets , 105 tablets, each tablet contains 5mg of <i>p</i> -nitrophenyl phosphate disodium salt with a balance of inert filler
34045	PNPP , 25g

Storage: Upon receipt store product at 4°C. Product is shipped at ambient temperature.

Introduction

Thermo Scientific™ PNPP (*p*-nitrophenyl phosphate disodium salt) is a widely used substrate for detecting alkaline phosphatase in ELISA-based applications. The reaction produces a water-soluble yellow product that absorbs light at 405nm.

Procedure for using PNPP in an ELISA

CAUTION: Avoid PNPP contact with eyes, skin, and clothing. Please see the MSDS for handling information.

1. Equilibrate PNPP to room temperature before opening.
2. Dilute the Diethanolamine Substrate Buffer from 5X to 1X.
3. Dissolve 10mg of PNPP or two PNPP Tablets in 10mL of 1X Diethanolamine Substrate Buffer.

Note: Diethanolamine is effective at concentrations from 10mM to 1M.

4. Add 100µL of the PNPP solution to each microplate well. Incubate plate at room temperature for 30 minutes or until sufficient color develops.
5. To stop the reaction, add 50µL of 2N NaOH to each well.
6. Measure the absorbance at 405nm. Alternatively, monitor reaction in a kinetic ELISA reader.

Related Thermo Scientific Products

31493	EZ-Link™ Maleimide Activated Alkaline Phosphatase Kit
15075	Reagent Reservoirs, 200/pkg
15082	Microtube Racked System, 960 tubes
15036	Sealing Tape for 96-Well Plates, 100/pkg
37621	1-Step™ PNPP, 100mL
21323	Streptavidin, Alkaline Phosphatase Conjugated, 3mg
31002	NeutrAvidin™ Protein, Alkaline Phosphatase Conjugated, 2mg

General References

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- Cordell, J.L., et al. (1984). Immunoenzymatic labeling of monoclonal antibodies using immune complexes of alkaline phosphatase and monoclonal anti-alkaline phosphatase. *J Histochem Cytochem* **32**(2):219-29.
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- Harlow, E. and Lane, D. (1988). *Antibodies: A Laboratory Manual*. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y. pp. 56-100.
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- Yolken, R.H. (1982). Enzyme immunoassays for the detection of infectious agents in body fluids: current limitations and future prospects. *Rev Infect Dis* **4**:35-68.

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