

FT-B3AK00



INTERFINE CHEMICALS

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BIOSCIENCES

pNPP Tablets & Powder

p-NitroPhenyl Phosphate (pNPP), disodium salt

Alkaline phosphatase substrates for ELISA applications

Product Description

pNPP tabs, 20mg	B3AK00	24 Tabs	20 mg of pNPP
	B3AK01	100 Tabs	20 mg of pNPP
pNPP tabs, 5mg	UP89562H	100 Tabs	5 mg of pNPP
	UP89562F	1000 Tabs	5mg of pNPP
pNPP, UltraPure	80914C	25 g	Na salt, Hexahydrate
	809147	100 g	MW: 371.1 Biotech grade

Storage: +4°C (L) (for long term storage, its better to store the powder at -20°C (H))

Directions for use

The procedure is designed for standard ELISA techniques (96-well microplates), but can be adapted for other applications. Perform your immunoassay according your usual procedure before processing pNPP staining. At the end of the immuno- or cell assay, wells of microplates probed with Alkaline Phosphatase reagent (typically, AP-labeled secondary antibody or streptavidin) are usually washed 3 times with PBS + Tween20.

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. Weight desired amount (powder) or take tabs as needed – i.e. weight 10mg powder, or take 2 tabs of 5mg.
3. Dissolve 10mg of pNPP in 10mL of suitable substrate buffer .

Note: a standard assay buffer is 100 mM Tris-HCl, pH 9.5 , 100 mM NaCl , 5 mM MgCl₂.

Alternative buffers have been used:

- Diethanolamine solution at 10mM to 1M: 5.1M DEA, pH 9.8 [4] ; or 10mM DEA 0.5M Magnesium Chloride, pH9.5 [4].
- in cell preparations containing AP: 10 mM pNPP and 2 mM EDTA in 20 mM MES at pH 6.0 [4].

A standard concentration is 1mg pNPP/mL solution, but this may be optimized in your application.

Note: This working solution should be prepared just before use. Protect from light. Discard unused reagent..

4. Add 100µL (or 200 µl for #B3AK01) of the PNPP solution to each microplate well.

Incubate plate at at 37°C for 30 minutes or until sufficient color develops.

Note: room temperature incubation also works. It is thought to give less border signal variation, but weaker signal and less reproducible results from experiment to experiment.

5. (optional – end point assays) To stop the reaction, add 50µL of 2N NaOH to each well.
6. Measure the absorbance at 405nm with a standard microplate reader.

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- In end-point assays, pNPP incubation time may be optimized between 10 and 60 min. Wells are added or not with 100µl reaction stop solution (i.e. 1 N NaOH with 0.25 M EDTA), and color is measured at 405 nm.
- In kinetic assays, the formed yellow color appears rapidly and monitored in a kinetic ELISA reader at 405/650 nm.

Additional information

Our pNPP reagents are made of ultrapure material (>98% purity, <1% inorganic phosphate). pNPP is available as:

- powder**: ultrapure raw material for home made preparation and manufacturers
- pre-weighed tablets**: a convenient format to save time and reproducibility. Each tablet contains 5mg, 20mg or 30 mg of p-Nitrophenyl Phosphate, disodium hexahydrate. Balance is inert filler.
- ready-to-use solution** substrate (ask for product #UP66478): a high quality active and stable reagent which has been optimized for sensitive and accurate measurement of AP (Alkaline Phosphatase) activity in ELISA procedures.

pNPP is hydrolyzed rapidly in the presence of Alkaline Phosphatase to p-nitrophenol and inorganic phosphate. The formed p-nitrophenol has an intense yellow color that is measured at 405 nm. Making pNPP reagents for AP assays involves preparing a stock solution, that is stable for limited time, and dilution to final using concentration, that is stable for one day only. Unlike daily preparations, our pNPP tablets (and solution UP66478) avoid tedious weighing, hence saving time and providing more reproducible reagents, and also allows longer storage. Furthermore we have a pNPP ready-to-use one-component solution stable for at least 20 months (ask for item UP66478).

Related reagents

Other reagents for immunodetections using pNPP substrate:

- AP-labeled secondary antibodies (p.A324)
- AP labeled (strept)avidins #[UP518498](#) (p.A350)
- WB: ProTran 0.2µm NC membranes, 20x20cm, #[S31441](#)
Blotting paper 1mm thick, 460x570cm, #[BP2791](#)
Protein Membrane Reversible stain #[20078A](#)
- SeaBlock agent #[UP40301A](#)
- TBS buffer (Tris Buffer Saline) #[UP74004A](#)
- PBS buffer (Phosphate Buffer Saline) #[UP68723A](#)
- BSA #[UPQ84170](#) (powder) or #[UP900130](#) (soln 30%)
- BioBlock Saturating agent for blotting (inTBS) #[N13650](#)
- Non fat milk powder #[768701](#)

Other AP Substrates

- chromogenic pNPP solution #[UP66478](#), [UPS0817](#), [UPS0818](#) and kits #[BP708](#)
AP-Blue (490-650nm) #[CB013](#) and AP-Red (450-550nm) #[CB014](#)
- fluorigenic FDP powder and kit #[HT079](#), 4-MUP (, more sensitive than pNPP)
- chemiluminescent Luminometric Alkaline Phosphatase Assay Kit # JQ6760)

*Phosphate assay kit # CI4211 (based on molybdate and malachite green dye; 600-660nm reading)

Ordering information

Catalog size quantities and prices may be found at <http://www.interchim.com>.
Please inquire for higher quantities (availability, shipment conditions).
For any information, please ask : Uptima / Interchim; Hotline : +33(0)4 70 03 73 06

[Order on-line](#) or [Contact](#) your local distributor

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