

pNPP Solution (AP Substrate for ELISA)

Description

The *Uptima* pNPP substrate is a chromogenic substrate for the detection of alkaline phosphatase (AP), giving a yellow soluble by-product. It is classically used in ELISA techniques. The formulation is optimized for a long shelf-life and for an excellent lot to lot reproducibility. It is conditioned in a unique vial containing a mixture of p-NitroPhenyl Phosphate (pNPP) of high purity and stabilizers. Upon reaction with alkaline phosphatase conjugates, it gives an intense yellow color that can be read at 405 nm. Uptima offers this optimized formulation where accurate and sensitive results are required. This substrate is not suitable for immunochemistry or western blotting (ask Uptima for suitable alternative substrates).

Product Number: UP664790, 200ml UP664791, 500ml UP664792, 1L

Sensitivity *Uptima* pNPP substrate is very sensitive and generates very low background. *Uptima* pNPP generally outperforms other available AP substrates in most applications, and often similar chromogenic detection systems with peroxidase.

Stability The substrate is stable for more than 30 months at +4°C.

Storage +4°C protected from light. (L)
The pNPP substrate is sensitive to certain storage and usage conditions:

- Avoid direct light exposure, or contact with contaminating substances.
- Do not pipette directly from the bottle : fill first the desired solution in a clean container
- Do not leave the bottle open for too long.
- Ship with frozen gel packs. Do not freeze.

Suggested Protocol

Uptima pNPP is ready-to-use. Simply allow the desired amount to reach room temperature.

The ELISA assay may be performed according to your standard protocol. Wash the microplates thoroughly to remove unbound alkaline phosphatase labeled probe (antibody, lectin, nucleotide...). Insufficient washes may lead to undesired background. Uptima recommends 4 washes in PBST (NaCl 150mM, phosphate 20mM, Tween20® 0.1%, pH7.5).

Add 150 µl of pNPP substrate to each microplate well. Agitate slowly to homogenize.
Incubate for 30 mins at +37°C, protect from light. The color develops immediately.

Note : Uptima pNPP solution is ready-to-use. Do not dilute.

The volume per well and incubation duration may be adjusted depending on the detection system.

If the staining is too rapid or intense, primary or secondary antibodies should be diluted, or staining time reduced to 15 min. It is not recommended to dilute the pNPP substrate

The absorbance is read directly at 405 nm, or after a stop step (point 4) for more accurate results.

Do not compare values obtained with stopped and non-stopped.

Reaction stop (optional) : add 100 µl of stop solution (1 M NaOH) in each well. The color remains yellow, but does not fluctuate, allowing reading at 405nm within 2 hours or more.

Related reagents

- Other AP Substrates – chromogenic
chromogenic
pNPP powder ([UP89562C](#)), tablets (#[UP89562F](#), [UP73250](#)), and kits ([BP708](#))
AP-Blue [490-650nm] ([CB0131](#)) and AP-Red [450-550nm] ([CB0141](#))
- Other AP Substrates – fluorogenic
FDP powder and kit ([HT079](#))
- Other AP Substrates – chemiluminogenic
VisGlo AP Substrate ([BV3021](#), [BV3031](#)) UptiLight ECL ([UP996190](#), ([BM4961](#), [58372A](#)))
- See our [BioScience catalog](#) for AP-labeled secondary antibodies (page A324) and streptavidin (A350), buffers and saturants (A365)

Other HRP Substrates -

TMB solution ([UP664780](#))DAB tablets [732310](#)ADHP ([39423A](#), kit [HS6241](#))

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