
AP substrate, pNPP Solution

Product Description

The *Uptima* pNPP substrate is a chromogenic substrate for alkaline phosphatase (AP), giving a yellow soluble by-product. It is classically used in ELISA technics. The formulation is optimized for a long shelf-life and for an excellent lot to lot reproducibility. It is conditioned in a unic 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 colour that could be read at 405 nm. Uptima offers this optimized formulation where accurate and sensitive results are sought. This substrate is not suitable for immunochemistry or western blotting (ask Uptima for other substrates).

Cat. Number: UP664790, 200ml UP664791, 500ml UP664792, 1L

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

Stabilité The substrate is stable more than 20 months at +4°C.

Storage +4°C protected from light. (K)

The pNPP substrate is sensitive to certain storage and use conditions:

1. Avoid a direct light exposure, or contact with contaminating substances.
2. Do not pipet directly from the bottle : fill prealably the desired solution in a clean container (made of immunology grade plastic)
3. Do not leave the bottle open a too long time.
4. Ship with frozen gel packs. Do not freeze.

Directions for Use

Suggested Protocol

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

1. The ELISA assay may be performed according to your standard protocol. Wash the microplates thoroughly to remove unbound alkaline phosphatase labelled probe (antibody, lectin, nucleotide...). Insufficient washes may lead to undesired back ground. Uptima recommends 4 washes in PBST (NaCl 150mM, phosphate 20mM, Tween20@ 0.1%, pH7.5).
2. Add 150 µl of pNPP substrate to each microplate well. Agitate slowly to homogenize. Incubate for 30 mn at +37°C, protected from light. The colour 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 to rapid or intense, primary or secondary antibodies should be diluted, or staining time reduced to 15 min. It is not recommended at all to dilute the pNPP substrate*
3. The absorbance is read directly at 405 nm, or after a stop step (point 5) for more accurate results. Do not compare values obtained with and without reaction stop.
4. Reaction stop (optionnal) : add 100 µl of stop solution (1 M NaOH) in each well. The colour remains yellow, but do not fluctuate, allowing to read OD at 405nm within 2 hours or more.

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Contact your local distributor

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P.1/1