

## Product Information

### Alkaline Phosphate Yellow (pNPP) Liquid Substrate for ELISA

ready to use solution

Catalog Number **P7998**

Storage Temperature 2–8 °C

#### Product Description

In ELISA experiments that use alkaline phosphatase, *p*-nitrophenylphosphate (pNPP) is a widely used substrate.<sup>1-2</sup> This product is supplied as a ready-to-use buffered alkaline phosphatase substrate that contains pNPP. This proprietary formulation is an improvement over traditional formulations in that it includes an insoluble stabilizer. This stabilizer enhances stability and reactivity, and greatly reduces baseline absorption.

Prior to the reaction with alkaline phosphatase, the substrate should appear as a colorless to pale yellow solution. A yellow product will develop during the reaction with alkaline phosphatase in microwell applications (ELISA). For end-point assays, the reaction may be stopped with a NaOH solution. Since this substrate forms a soluble end-product, it is **not** recommended for blotting or histochemistry applications.

Several publications cite use of this product in their protocols.<sup>3-8</sup>

#### Precautions and Disclaimer

For R&D use only. Not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

#### Storage/Stability

Store the product at 2–8 °C. This substrate is light-sensitive and should be protected from direct sunlight or UV sources.

#### Procedure

- This ready-to-use product is supplied at the working concentration. Dilution of the substrate is **not** recommended. To reduce the intensity of a reaction, it is recommended that the antibodies or conjugates be diluted.
- The product should be brought to room temperature (~25 °C) before use. Decant or pipette the solution to separate it from the insoluble stabilizer pellets. If the product is vigorously agitated, some of the insoluble stabilizer may remain in suspension and can be easily filtered out.
- Following the reaction with alkaline phosphatase, a yellow reaction product forms that may be read at 405 nm. For end-point assays, the reaction can be stopped by the addition of a NaOH solution. Add 50 µL of 3 M NaOH for every 200 µL of substrate reaction. This also yields a yellow end product that can be read at 405 nm.

#### References

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3. Wittwer, A.J. *et al.*, *Biochemistry*, **46(21)**, 6393-6401 (2007).
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7. Yang, W.H. *et al.*, *Cell Host Microbe*, **24(4)**, 500-513 e5 (2018).
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