

Product Information

Phosphatase Substrate

Powder

P4744

Product Description

Synonyms (substrate): 4-Nitrophenyl phosphate disodium salt hexahydrate, *p*-nitrophenyl phosphate disodium salt hexahydrate, pNPP disodium salt hexahydrate

CAS Registry Number (pNPP hexahydrate):
333338-18-4

Molecular Formula (pNPP hexahydrate):
 $C_6H_4NO_6PNa_2 \cdot 6H_2O$

Formula Weight (pNPP hexahydrate): 371.14

p-Nitrophenyl phosphate (pNPP) is a soluble substrate for use with alkaline phosphatase conjugates in ELISA procedures.¹⁻³ pNPP may also be used to determine alkaline and acid phosphatase activity in physiological fluids and other aqueous solutions. This substrate produces a soluble end product that is yellow in color and can be read spectrophotometrically at 405 nm. The pNPP reaction may be stopped with 3 M NaOH solution and read at 405 nm.

Several theses⁴ dissertations⁵⁻¹⁰ have cited use of product P4744 in their research protocols.

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

These capsules should be stored at -20 °C.

Solubility

This product is tested for solubility in water at 100 mg/mL.

Preparation Instructions

Dissolve contents of capsules to the desired concentration in either of the following buffers:

- 0.1 M glycine (pH 10.4), with 1 mM MgCl₂ and 1 mM ZnCl₂
- 1 M diethanolamine (pH 9.8), with 0.5 mM MgCl₂

Typically a pNPP working stock concentration of 1 mg/mL is prepared.

Glycine Buffer

To prepare 0.1 M glycine buffer (pH 10.4), with 1 mM MgCl₂ and 1 mM ZnCl₂:

1. Add 7.51 g of glycine, 203 mg of MgCl₂, and 136 mg of ZnCl₂ to ~980 mL of water. Mix.
2. Adjust pH to 10.4 with 19 M NaOH.
3. Adjust the volume to 1 L with water.

Diethanolamine Buffer

To prepare 1 M diethanolamine buffer (pH 9.8), with 0.5 mM MgCl₂:

1. Add 97 mL of diethanolamine and 100 mg of MgCl₂ to 800 mL of water. Mix.
2. Adjust pH to 9.8 with 10 M HCl.
3. Adjust the volume to 1 L with water.

Procedure

General ELISA procedure with alkaline phosphatase conjugates

1. Add 200 µL of substrate solution (typically 1 mg/mL) per well.
2. Incubate the plate in the dark for 30 minutes at room temperature.
3. The absorbance can be read at 405 nm on a multiwell plate reader.
4. The reaction may be stopped by adding 50 µL of 3 M NaOH per 200 µL of reaction mixture.

Related Products

p-Nitrophenol is the hydrolysis product of *p*-nitrophenyl phosphate (pNPP) and may be used as a standard to determine enzyme activity. It has a formula (C₆H₅NO₃) weight of 139.1.

- Standard solutions can be prepared from the powdered product (Cat. No. 1048) in 0.02 to 1 M NaOH solution.
- A 10 mM *p*-nitrophenol solution (Cat. No. N7660) is also available.

References

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3. Voller, A., and Bidwell, D., "Enzyme-linked immunosorbent assay", in *Manual of Clinical Laboratory Immunology*, 3rd ed. (Rose, N.R. *et al.*, eds.). American Society for Microbiology (Washington, D.C.), pp. 99-109 (1986).
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5. Crowley, Katherine F., "Mosses Influence Vascular Plants in Rich Fens Through Effects on the Biogeochemistry of Shallow Soils". Cornell University, Ph.D. dissertation, p. 10 (2009).
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