Product Information

Phosphatase Substrate

40 mg tablets

P5994

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): $C_6H_4NO_6PNa_2 \bullet 6H_2O$

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.

This product consists of formulated tablets with 40 mg of pNPP per individual tablet. Several theses[4-7] and dissertations[8-10] have cited use of product P5994 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 tablets should be stored at –20 °C.

Preparation Instructions

Dissolve tablets to the desired concentration in either of the following buffers:

- 0.1 M glycine (pH 10.4), with 1 mM $MgCl_2$ and 1 mM $ZnCl_2$
- 1 M diethanolamine (pH 9.8), with 0.5 mM $MgCl_2$

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

Glycine Buffer

To prepare 0.1 M glycine buffer (pH 10.4), with 1 mM $MgCl_2$ and 1 mM $ZnCl_2$:

1. Add 7.51 g of glycine, 203 mg of $MgCl_2$, and 136 mg of $ZnCl_2$ 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 $MgCl_2$:

1. Add 97 mL of diethanolamine and 100 mg of $MgCl_2$ 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 ($C_6H_5NO_3$) 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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5. Van Poelvoorde, Laura, "Calcium carbonate-based particles and capsules for therapeutic enzymes". Universiteit Gent, M.Sc. thesis, p. 35 (2017).
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8. Antoñanzas, Román Pérez, "New Processing and Applications of Collagen Containing Calcium Phosphate Cements". Universitat Politècnica de Catalunya, Ph.D. dissertation, p. 125 (2011).
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10. Langenberg, Marijke C.C., "Controlled human infection models as a tool for malaria and schistosomiasis vaccine research". Universiteit Leiden, Ph.D. dissertation, p. 127 (2021).

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