Product Information

Phosphatase Substrate

Preweighed 40 mg capsules

P5744

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 capsules formulated with 40 mg of pNPP per individual capsule. Several theses[4-6] and dissertations[7,8] have cited use of product P5744 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.

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 $MgCl_2$ 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

1. Voller, A. *et al*., *Bull. World Health Organ*., **53(1)**, 55-65 (1976).
2. Engvall, E., *Methods Enzymol*., **70(A)**, 419-439 (1980).
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).
4. Hsu, Ching-Yin, "Application of Silver Nanoparticle-based Materials in Orthopedic Surgery". University of California Los Angeles, M.S. thesis, p. 8 (2014).
5. Dawkins, Dionne, "Calibration and validation of an *in vitro* ultrasound device to deliver targeted thermal enhancement of hMSC osteogenesis". City College of New York, M.S. thesis, p. 56 (2015).
6. Mechtler, Katharina, "In-vitro-Charakterisierung neuer Polymer- und Keramikmaterialien für Knochenersatzimplantate" ("*In vitro* characterization of new polymer and ceramic materials for bone replacement implants"). Universität Wien, Mag.pharm. thesis, p. 30 (2017).
7. Maloney, John Mapes, "Chemomechanics of Attached and Suspended Cells". Massachusetts Institute of Technology, Ph.D. dissertation, p. 171 (2012).
8. Abushahba, Ahmed, "Craniomaxillofacial Bone Tissue Engineering – A Translational Approach". University of Helsinki, Ph.D. dissertation, p. 52 (2021).

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