Product Information

Phosphatase Substrate

Preweighed 100 mg capsules

P5869

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 100 mg of pNPP per individual capsule. Several dissertations[4-6] have cited use of product P5869 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. O'Reilly, Isobel, "Potentiation of Drug-Induced Cytotoxicity by Conjugated Linoleic Acids (CLA) in *In Vitro* models of Drug-Resistant Cancer". Dublin City University, Ph.D. dissertation, p. 49 (2009).
5. Vallejo, Catalina Estrada, "Characterization of Genetically Modified HUCPVCs as an Osteogenic Cell Source". University of Toronto, Ph.D. dissertation, p. 157 (2013).
6. Mang, Tanja, "Evaluation of the therapeutic potential of GDF5 mutants to treat osteoarthritis". Technischen Universität Darmstadt, Dr. rer. nat. dissertation, p. 34 (2018).

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