

Product Information

4-Nitrophenyl phosphate bis(cyclohexylammonium) salt

1

Phosphatase substrate

N3129

Product Description

CAS Registry Number: 52483-84-8

Synonyms: pNPP, p-Nitrophenyl Phosphate,

pNPP bis(cyclohexylammonium) salt

Molecular Formula: $C_6H_4NO_6P \bullet (C_6H_{11}NH_3^+)_2$

Formula Weight: 417.44

 λ_{max} : 311 nm¹

Extinction Coefficient: $E^{mM} = 9,867$ (in 0.01 N NaOH)

Storage Temperature: -20 °C

4-Nitrophenyl Phosphate (pNPP) is the substrate of choice for use with alkaline phosphatase conjugates in Enzyme Linked Immunosorbant Assay (ELISA) procedures, due to its high sensitivity. ^{2,3} ELISA applications that use pNPP may be read in timed assays or stopped with alkaline solutions for delayed readings. ⁴ 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 the addition of 3 M NaOH solution and read at 405 nm.

Several publications⁵⁻⁸ and theses^{9,10} have cited use of Product Number N3129 in their research protocols.

Precautions and Disclaimer

This product is 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.

Preparation Instructions

Dissolve pNPP in either:

- 0.1 M glycine buffer that contains 1 mM MgCl₂, 1 mM ZnCl₂, pH 10.4, or
- 1 M diethanolamine buffer containing 0.5 mM MgCl₂, pH 9.8, to the desired concentration.
- Typically a pNPP concentration of 1 mg/mL is used.

Procedure

General ELISA procedure with alkaline phosphatase conjugates

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

Troubleshooting

If the background is too high:

- 1. Use a blocking step prior to the application of the primary antibody. Normal Serum (5% v/v) from the same species as the host of the second antibody generally produces the best results.
- 2. Additional blocking agents for an ELISA are:
 - 0.05% TWEEN® 20 in 50 mM TBS, pH 8.0.
 - 1% BSA containing 0.05% TWEEN® 20 in 50 mM TBS, pH 8.0.
 - 3% nonfat-dried milk in 0.01 M TBS (Cat. No. P2194). Do not use milk as a blocking agent when using avidin-biotin systems.



- 3. Use 0.05% TWEEN® 20 in all washing and antibody diluent buffers.
- 4. Run control wells without the primary antibody to check for non-specific reactivity of the secondary antibody/alkaline phosphatase conjugate.
- 5. Adjust the titer of the primary antibody and/or the alkaline phosphatase conjugate to determine the optimal working dilutions.

If no color develops or color is too faint:

- 1. Adjust the concentration of the primary antibody.
- 2. Adjust the concentration of the secondary antibody/alkaline phosphatase conjugate.
- 3. Determine if the enzyme conjugate is active by mixing a small sample of substrate and conjugate together in a test tube.
- 4. Increase the substrate incubation time or temperature.
- 5. Adjust the concentration of the coating antigen.
- Consider using an amplifying system such as avidin-biotin.

References

- Bowers, G. et al., Clin. Chem., 27(1), 135-143 (1981).
- 2. Voller, A. et al., Bull. World Health Organ., **53(1)**, 55-65 (1976).
- 3. Engvall, E., *Methods Enzymol.*, **70(A)**, 419-439 (1980).
- 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).
- 5. Bauerle, W. L. et al., J. Amer. Soc. Hort. Sci., **131(2)**, 295-301 (2006).
- Dahabreh, Z. et al., World J. Stem Cells, 6(4), 497-504 (2014).
- 7. Ásgeirsson, B. *et al.*, *Biochem. Biophys. Rep.*, **24**, 100830 (2020).
- 8. Jenkins, B. R. *et al.*, *FASEB J.*, **35(6)**, e21551 (2021).

- Inman, William Wright, III, "Hydraulic resistance: a determinant of short term stomatal conductance signaling in disparate xylem anatomy of red maple (Acer rubrum L.) and Shumard Oak (Quercus shumardii Buckl.)". Clemson University, M.S. thesis, p. 10 (2007).
- 10. D'Abadia, Patrícia Lima, "Tratamentos alternativos para a cicatrização de feridas: potencial regenerativo e atividade enzimática da fração soro do látex de *Hancornia speciosa* Gomes" ("Alternative treatments for wound healing: regenerative potential and enzymatic activity of the serum fraction of latex of *Hancornia speciosa* Gomes"). Universidade Estadual de Goiás, M.S. thesis, p. 75 (2021).

Notice

We provide information and advice to our customers on application technologies and regulatory matters to the best of our knowledge and ability, but without obligation or liability. Existing laws and regulations are to be observed in all cases by our customers. This also applies in respect to any rights of third parties. Our information and advice do not relieve our customers of their own responsibility for checking the suitability of our products for the envisaged purpose.

The information in this document is subject to change without notice and should not be construed as a commitment by the manufacturing or selling entity, or an affiliate. We assume no responsibility for any errors that may appear in this document.

Technical Assistance

Visit the tech service page at SigmaAldrich.com/techservice.

Terms and Conditions of Sale

Warranty, use restrictions, and other conditions of sale may be found at <u>SigmaAldrich.com/terms</u>.

Contact Information

For the location of the office nearest you, go to SigmaAldrich.com/offices.

The life science business of Merck operates as MilliporeSigma in the U.S. and Canada.

Merck and Sigma-Aldrich are trademarks of Merck KGaA, Darmstadt, Germany or its affiliates. All other trademarks are the property of their respective owners. Detailed information on trademarks is available via publicly accessible resources.

