

Product Information

SIGMAFAST™ *p*-Nitrophenyl Phosphate Tablets

Tablet, to prepare 5 mL

N1891

Product Description

Synonyms: pNPP tablets, *p*-Nitrophenyl Phosphate tablets, Phosphatase substrate tablets

SIGMAFAST™ *p*-Nitrophenyl phosphate (pNPP) tablets have been developed for use as a soluble substrate for the detection of alkaline phosphatase activity in Enzyme Immunoassays (EIA and ELISA assays).^{1,2} pNPP is the EIA/ELISA substrate of choice in alkaline phosphatase systems, as it exhibits high sensitivity. EIA/ELISA applications with pNPP may be read in timed assays, or stopped with alkaline solutions for delayed readings. SIGMAFAST™ pNPP tablets require no additional buffers to prepare an active substrate solution.

One pNPP tablet and one Trizma® Buffer tablet, dissolved in 5 mL of water, provides 5 mL of ready-to-use substrate. Each SIGMAFAST™ pNPP tablet set yields 5 mL of a solution that contains:

- 1.0 mg/mL pNPP
- 0.2 M Trizma® buffer
- 5 mM Magnesium chloride

Various publications have cited use of this product to report alkaline phosphate activity in ELISA,³⁻⁷ as well as in binding assays,⁸ and studies of hydrogels⁹ and of receptors.¹⁰ Several theses^{11,12} and dissertations¹³⁻²² have cited use of Cat. No. N1891 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.

Storage/Stability

The tablets should be stored at -20 °C.

Preparation Instructions

1. Remove the required number of pNPP and Trizma® Buffer tablets for assay. Return the box to the freezer.
2. Allow the tablets to warm to room temperature.
3. Open an equal number of pNPP tablet packages (silver foil) and Trizma® Buffer tablet packages (gold foil).
4. Drop the tablets into an appropriate container containing 5 mL of water for each tablet set. **Do not touch the tablets with your fingers.**
5. Vortex the solution until the tablets completely dissolve.

The SIGMAFAST™ pNPP substrate solution is now ready for use. For best results, the solution should be used within one hour.

Procedure

1. After the plate has been incubated with an alkaline phosphatase conjugate (generally 1-2 hours), wash thoroughly to remove unbound conjugate.
2. Add 200 µL of pNPP substrate solution to each well. Incubate the plate in the dark for ~30 minutes at room temperature.
3. After the incubation period, read the plate at 405 nm on a multi-well plate reader.
4. If the plate cannot be read immediately, add 50 µL of 3 N NaOH solution per 200 µL of reaction mixture.
5. Read the absorbance for the stopped reactions at 405 nm.

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.
6. Consider using an amplification system such as avidin-biotin.

References

1. Voller, A. *et al.*, *Bull. World Health Organ.*, **53(1)**, 55-65 (1976).
2. Jones, J.V. *et al.*, *J. Immunol. Methods*, **118(1)**, 79-84 (1989).
3. Bahouth, S.W., *Methods Mol. Biol.*, **126**, 281-299 (2000).
4. Arnold, J.N. *et al.*, *J. Biol. Chem.*, **280(32)**, 29080-29087 (2000).
5. Palmberger, D. *et al.*, *Biotechnol. J.*, **9(9)**, 1206-1214 (2014).
6. Morello, C.S. *et al.*, *J. Virol.*, **79(1)**, 159-175 (2005).
7. Nakamura, F. *et al.*, *J. Neurosci.*, **37(30)**, 7125-7139 (2017).
8. Chehola, R.W. *et al.*, *ACS Chem. Biol.*, **10(3)**, 844-854 (2015).
9. Castillo Diaz, L.A. *et al.*, *J. Tissue Eng.*, **5**, 2041731414539344 (2014).
10. Mygind, K.J. *et al.*, *J. Biol. Chem.*, **293(21)**, 8077-8088 (2018).
11. Kim, Margaret, "Comparison of Ovine Mesenchymal Cell and Osteoblast Osteogenic Capacity in 2D and 3D". Queensland University of Technology, M.Eng. thesis, p. 25 (2014).
12. Nicholls, Emma, "Etablering av forhold for stabil antistoffproduksjon fra EBV-transformerte humane B-celler" ("Establishment of conditions for stable antibody production from EBV-transformed human B cells"). Norges Arktiske Universitet, M.Sc.med. thesis, p. 7 (2018).
13. Nahrgang, Stefan, "Influence of Cell-Line and Process Conditions on the Glycosylation of Recombinant Proteins". École Polytechnique Fédérale de Lausanne, Ph.D. dissertation, p. 41 (2002).
14. Schmidmayr, Monika, "*In vitro* Untersuchungen zur Beeinflussung von Proliferation und Differenzierung humaner Osteoblasten durch Konzentration und Zyklizität von Progesteron nach Estradiolexposition" ("*In vitro* studies on the influence of progesterone concentration and cyclicity on proliferation and differentiation of human osteoblasts after estradiol exposure"). Technischen Universität München, Dr. med. dissertation, p. 9 (2008).
15. Fei, Zhengzheng, "Membrane Sandwich Electroporation for *In Vitro* Gene Delivery". The Ohio State University, Ph.D. dissertation, p. 44 (2009).
16. Yu, Deqiang, "Application of Membrane Chromatography in Bioprocessing". The Ohio State University, Ph.D. dissertation, p. 44 (2009).
17. Engebret, Iris Inge, "Nachweis onkogener humaner Papillomaviren im Oropharynx und an der Cervix uteri" ("Detection of oncogenic human papillomaviruses in the oropharynx and on the cervix uteri"). Universität zu Köln, Dr. med. dissertation, p. 32 (2013).

18. Taylor, Anna Rose, "The Effects of Maternal Energy Restriction During Mid-Gestation on Growth Performance, Immune Function, and Gene Expression in the Resultant Beef Offspring". South Dakota State University, Ph.D. dissertation, p. 125 (2014).
19. Hoac, Betty, "Enzymatic modifications of osteopontin and their role in mineralization". McGill University, Ph.D. dissertation, p. 138 (2018).
20. Townsend, Catherine Louise, "Characterisation of Naïve and Antigen-Experienced Human Antibody Repertoires". King's College London, Ph.D. dissertation, p. 134 (2018).
21. Nga, Tran Vu Thieu, "The Humoral Response against *Salmonella Typhi* Protein Antigens During Acute, Convalescent, and Chronic Typhoid Fever". The Open University, Ph.D. dissertation, p. 57 (2019).
22. Hefele, Lisa, "Epidemiological and serological investigations of vaccine-preventable diseases and their implications for vaccination policy in the Lao People's Democratic Republic". Universität des Saarlandes, Dr. rer. nat. dissertation, p. 24 (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 SigmaAldrich.com/terms.

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.

© 2018-2023 Merck KGaA, Darmstadt, Germany and/or its affiliates. All Rights Reserved.

N1891pis Rev 01/23 CMH,RBG,GCY,MAM

The Merck logo, consisting of the word "MERCK" in a bold, red, sans-serif font.