Product Information

SIGMA*FAST*™ *p*-Nitrophenyl Phosphate Tablets

Tablet, to prepare 20 mL

N2770

Product Description

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

SIGMA*FAST*™ *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. SIGMA*FAST*™ pNPP tablets require no additional buffers to prepare an active substrate solution.

One pNPP tablet and one Trizma® Buffer tablet, dissolved in 20 mL of water, provides 20 mL of ready-to-use substrate. Each SIGMA*FAST*™ pNPP tablet set yields 20 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,[3-10] as well as in general alkaline phosphatase assays.[11-12] Several theses[13-15] and dissertations[16-24] have cited use of N2770 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 20 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 SIGMA*FAST*™ 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 multiwell 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. Honda, Y. *et al*., *Vaccine*, **16(13)**, 1276-1282 (1998).
4. Tibbett, M. *et al*., "Methods for Determining Extracellular and Surface-Bound Phosphatase Activities in Ectomycorrhizal Fungi", in *Mycorrhiza Manual* (A. Varma, ed.). Springer-Verlag (Berlin / Heidelberg), Chapter 14, pp. 217-225 (1998).
5. Roman, M. *et al*., *Methods Mol. Med*., **29**, 451-472 (2000).
6. Dean, G.S., and Isenberg, D.A., *Clin. Exp. Rheumatol*., **19(3)**, 251-257 (2001).
7. Permar, S.R. *et al*., *J. Virol*., **77(7)**, 4396-4400 (2003).
8. Arnold, J.N. *et al*., *J. Biol. Chem*., **281(11)**, 6955-6963 (2006).
9. Jarousse, N. *et al*., *J. Immunol*., **187(11)**, 5540-5547 (2011).
10. Passmore, C. *et al*., *Hum. Vaccine Immunother*., **10(3)**, 615-622 (2014).
11. Howitt, S.M., *Biochem. Mol. Biol. Educ*., **35(5)**, 350-354 (2007).
12. Wang, L. *et al*., *Plant Physiol*., **157(3)**, 1283-1299 (2011).
13. Win, Pei Lim, "Development Of An Enzyme-Linked Immunosorbent Assay (ELISA) For The Detection Of Pistachio Residues In Processed Foods". University of Nebraska – Lincoln, M.S. thesis, p. 60 (2010).
14. Robinson, Kevin, "Characterizing the Immune Function of the Brown Bullhead (*Ameiurus nebulosus*) from Less Contaminated and Highly Contaminated Locations along the Detroit River". University of Waterloo, M.Sc. thesis, p. 35 (2011).
15. Faustini, Siân Elizabeth, "How Does CD8 Loss Impact the Immune Response to Systemic Salmonella Infection?". University of Birmingham, M.Sc. thesis, p. 31 (2013).
16. McGowan, Jacqueline Ellen, "Effect of Body Condition Score at Calving on Adaptive Immune Function During Early Lactation in New Zealand Dairy Cows: Effect of calving body condition on adaptive immune function". University of Waikato, M.Sc. thesis, p. 71 (2014).
17. Caliebe, Janina, "Adsorption von Plasmaproteinen an modifizierten Fremdoberflächen - Bedeutung für die Hämokompatibilität der extrakorporalen Zirkulation" ("Adsorption of plasma proteins on modified foreign surfaces - importance for the hemocompatibility of the extracorporeal circulation"). Eberhard-Karls-Universität zu Tübingen, Dr. med. dissertation, p. 28 (2005).
18. Erkkola, Maijaliisa, "Diet in Early Life and Antibody Responses to Cow's Milk and Type 1 Diabetes Associated Autoantigens". National Public Health Institute (Helsinki) and University of Tampere, Ph.D. dissertation, p. 68 (2005).

19. Corsi, Karin A., "The Effect of Bmp4 and Mechanical Stimulation on Muscle-Derived Stem Cells: Implications for Bone and Articular Cartilage Regeneration". University of Pittsburgh, Ph.D. dissertation, pp. 20, 52, 97 (2007).
20. Reuter, Richard Ryan, "Nutritional modulation of the immune system of beef cattle". Texas Tech University, Ph.D. dissertation, p. 143 (2007).
21. Kubitzki, Tina, "Development of a Process for the Cleavage of a Mucin Fusion Protein by Enterokinase". Bielefeld University, Dr. rer. nat. dissertation, p. 122 (2008).
22. Márta, Dóra, "Molecular Monitoring of Meat Spoiling *Pseudomonas* Species and Analysis of Staphylococcal Enterotoxin Expression and Formation". Corvinus University of Budapest, Ph.D. dissertation, p. 45 (2011).
23. Taema, Maged M., "The *in vitro* characterisation of prion diseases of sheep". University of Nottingham, Ph.D. dissertation, p. 40 (2012).
24. Cox, Helen C., "A biodegradable system for the tailored delivery of growth factors and its application to bone morphogenetic protein-2 delivery for bone repair". University of Nottingham, Ph.D. dissertation, pp. 163, 164, 271 (2013).
25. Alrashidi, Hanan, "The Interplay of Complement Proteins C1q and Factor H". University of Leicester, Ph.D. dissertation, pp. 30, 39, 43, 45, 47 (2014).
26. Cook, Sarah, "The Role of CC-Chemokine Receptor-Like 2 in the B cell Response". University of Birmingham, Ph.D. dissertation, p. 43 (2014).
27. Zeadin, Melec Ghiulnas, "Investigating the role of leptin and GSK-3 in the osteogenic differentiation of vascular smooth muscle cells". McMaster University, Ph.D. dissertation, p. 131 (2014).
28. Abbow, Hussein, "Studies on ligand interactions of human complement factor H". University of Leicester, Ph.D. dissertation, pp. 30, 34 (2016).
29. Moore, Shona, "An Analysis of the Structure and Function of Malarial Duffy-Binding-Like Protein Domains using Recombinant Fusion Proteins". University of Warwick, Ph.D. dissertation, p. 45 (2016).
30. Buang, Norzawani Binti, "Genetic and cellular mechanisms of autoimmunity in lupus-prone B6.129- mice". Imperial College London, Ph.D. dissertation, p. 92 (2017).
31. Hooton, Diane Frances, "Brush border digestion: Development of a physiologically relevant *in vitro* model". Massey University, Ph.D. dissertation, p. 3-77 (2018).
32. Tu, Huy, "Determinants Of Antibody Immunity In Flavivirus Infections And Immunization". University of Vermont, Ph.D. dissertation, p. 130 (2021).
33. Sarapulov, Alexey, "B Cell-Mediated Immunity and Signal Regulation: The roles of MIM/MTSS1 and IRF4". University of Turku, Ph.D. dissertation, p. 76 (2022).

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