

Product Information

Phosphatase Substrate

Suitable for manufacturing of diagnostic kits and reagents

SRE0026

Product Description

Synonyms: 4-Nitrophenyl phosphate disodium salt hexahydrate, *p*-nitrophenyl phosphate disodium salt hexahydrate, pNPP disodium salt hexahydrate

Molecular Formula: $C_6H_4NO_6PNa_2 \cdot 6H_2O$

Formula Weight: 371.14

p-Nitrophenyl phosphate (pNPP) is a substrate used with alkaline phosphatase conjugates in such applications as ELISA procedures. Hydrolysis of pNPP produces *p*-nitrophenol, a soluble end product. The pNPP reaction is stopped with the addition of 3 M NaOH solution and the intensity of the yellow color is determined spectrophotometrically at 405 nm.

In physiological samples and model studies, pNPP has been used to determine alkaline phosphatase¹⁻³ and acid phosphatase activity.^{4,5} Other applications that use pNPP include studies of cytokine levels.⁶

Several research publications^{7,8} and an academic thesis⁹ have cited use of product SRE0026 in their research protocols.

Precautions and Disclaimer

For further (non-TSCA-only use in the US) manufacturing uses only. Not intended for direct use in humans or animals. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

This product has a recommended retest date of one year when properly stored at -20 °C.

Preparation Instructions

A pNPP solution with a concentration of 1 mg/mL is typically used. Initial stock solutions of pNPP can be prepared in either of two 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 $MgCl_2$

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.

The alkaline phosphatase reaction in a multiwell plate may be stopped by adding 50 μ L of 3 M NaOH solution per 200 μ L of reaction mixture.

References

1. Dulis, B., and Wilson, I. B., *Cancer Res.*, **38(8)**, 2519-2522 (1978).
2. Koo, H. C. *et al.*, *Clin. Vaccine Immunol.*, **11(6)**, 1070-1074 (2004).
3. Bel-Ochi, N. C. *et al.*, *J. Immunol. Methods*, **394(1-2)**, 107-114 (2013).
4. Foti, A. G. *et al.*, *Clin. Chem.*, **23(1)**, 95-99 (1977).
5. Laidler, P. *et al.*, *Biochem. Mol. Biol. Educ.*, **32(6)**, 400-409 (2004).
6. Böttcher, M. F. *et al.*, *Pediatr. Res.*, **47(1)**, 157-162 (2000).
7. Forrelad, M. A. *et al.*, *Front Microbiol.*, **11**, 570794 (2020).
8. Tang, C.-C. *et al.*, *eLife*, **10**, e67772 (2021).
9. Masilela, Charity Mandisa, "Metabolic Bioactivity of *Athrixia phyllicoides*". University of Zululand, M.Sc. thesis, p. 28 (2016).

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